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PHOTODYNAMIC THERAPY TO THE ENDOMETRIUM AS A PRIMARY TREATMENT MODALITY FOR MENORRHAGIA.

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**Thesis submitted for the degree of Doctor of Medicine in the
University of London.**

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ABSTRACT

Menorrhagia is a condition which affects a considerable number of women. The traditional treatment is hysterectomy, which has significant morbidity associated with it. Over recent years more minimally invasive techniques have been developed to treat menorrhagia without resorting to hysterectomy.

Photodynamic therapy (PDT) is a non-thermal technique which can be used to cause tissue damage. It requires the activation of a photosensitiser with light, which in the presence of oxygen produces cytotoxic oxygen species.

5-aminolaevulinic acid (ALA) is a photosensitising agent which has been used as a pre-cursor for the photoactive protoporphyrin IX (PPIX) in the past to successfully destroy the endometrium in the rat and rabbit model. When the technique has been used in humans, the effect has been unsatisfactory and has not been reproducible.

This thesis demonstrates that by the addition of an iron chelator CP94 there is a significantly increased level of PPIX in the endometrium, as measured with fluorescence microscopy after both chemicals are instilled into the uterine cavity of the rabbit. We show that pH has little effect on these data. The optimum time for PDT was shown to be seven hours after instillation. We show a dramatically increased PDT effect when using a combination of ALA and CP94 in the rabbit endometrium. In addition this PDT effect remains at 28 days. These studies showed that it may be possible to improve on the previously reported disappointing clinical results using ALA-PDT by the addition of the iron chelating agent, CP94.

We performed preclinical trials of light distribution within the human uterine cavity using different light delivery systems. This showed that an effective light dose could be applied to the irregular shaped endometrial cavity, in order to maximise the PDT effect in humans. The technique is now ready to take into pilot clinical studies.

STATEMENT OF ORIGINALITY

The work presented in this thesis has been carried out at the National Medical Laser Centre and the Department of Gynaecology at University College London Hospitals. The work was carried out by myself and with the collaboration of others in the department, and has not been entered for a higher degree or award at this or any other university. The majority of the work involves original ideas and observations as to the effect of photodynamic therapy on the normal endometrium, using the topical administration of the photosensitiser aminolaevulinic acid and using a novel iron-chelator agent (CP94). I hope that this work will make a substantial contribution to the scientific understanding and clinical practice of local destruction of the endometrium using photodynamic therapy.

All work on human tissue was carried out under consultant gynaecologist supervision, and was given ethical approval by the Joint UCL/UCLH Committees On The Ethics Of Human Research. All women participated freely and consented to having their uterus used after hysterectomy.

HOME OFFICE ARRANGEMENTS

All animal experiments were carried out under licence from the Home Office.

The experiments were carried out under supervision of the animal house superintendent. The author holds a personal licence PIL 70/14404, and project licence PPL 70/3911 under the Animals (Scientific Procedures) Act 1986, valid for work carried out at University College London.

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ABBREVIATIONS

ALA	5-aminolaevulinic acid
AlS2Pc	Aluminium disulphonated phthalocyanine
au	arbitrary units
CCD	Charge coupled device
CP94	1,2-diethyl-3-hydroxypyridin-4-one
CT	Computerised tomography
DHE	Dihaematoporphyrin ester/ether
EDTA	Ethylene diamine tetra-acetic acid
Fe ²⁺	Ferrous iron
Fe ³⁺	Ferric iron
H&E	Haematoxylin and eosin
HpD	Haematoporphyrin derivative
iv	Intravenous
LASER	Light amplification by the stimulated emission of radiation
MBL	Menstrual blood loss
MRI	Magnetic resonance image
mTHPC	meta tetra hydroxyphenyl chlorin
¹ O ₂	Singlet oxygen
PDT	Photodynamic therapy
PPIX	Protoporphyrin IX

The treatment, which I have described, seems to have proved its value, and there is every reason to give it the place that it deserves in therapeutics, a place which it is at present still far from having obtained, doubtless owing to its strangeness and unintelligibility. In reality, its scientific basis is much better and more solid than that of many other methods of medical treatment.

Niels Rydberg Finsen, 1901.
(The founder of phototherapy: 1860-1904).

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SECTION ONE

INTRODUCTION

CHAPTER 1: MENORRHAGIA

1.1 Introduction.

Menstruation is a normal physiological process in women whereby the endometrium is sloughed off if a pregnancy has not been achieved. Its monthly appearance commences at the menarche and finishes at the menopause. The concept of a monthly cycle is a modern phenomenon, as in primitive societies 'marriage' occurred at menarche or soon after, followed by a succession of pregnancies and periods of amenorrhoea associated with lactation. Finally, death often occurred before the menopause (Short 1976). In our modern western society with an average of two children per family, a short period of breast-feeding and longer life expectancy, women may only have a few months in their reproductive life when they do not menstruate. The age of menarche is also declining. In the mid-nineteenth century the average age was 17; whereas in the mid-twentieth century it was 13 years of age (Tanner 1962).

Normal menstrual blood loss (MBL) was defined in the 1960's in Sweden (Hallberg et al 1966). By careful measurement of total menstrual loss, they found that the mean loss to be 43 mls and the 90th centile of loss was found to be 80mls. They also found that where the loss was more than 60mls, women were at risk of developing anaemia. Cole et al found similar results with a mean loss of 37.5 mls (Cole et al 1971).

Therefore an average woman today may have 350-400 menses in their life, and lose 15-20 litres of blood throughout her menstrual life. However these figures are of little value as the quantity of blood loss cannot be easily

measured, and it is the woman's perception of the loss that is of relevance. A simpler method of assessing MBL is the presence, or otherwise of clots. The commonest objective indicator of heavy menstrual loss is iron deficiency anaemia. A low serum ferritin is more sensitive, although the test is rarely used (Lewis 1982).

In Hallberg's study, one quarter of women who had a blood loss in the normal range stated that their periods were heavy, and 40% of those with blood loss in excess of 80mls described their loss as 'light'. Gath et al reported that almost one third of over 500 women surveyed stated that their periods were heavy or very heavy. In addition 22% of their survey stated that the blood loss was of such an extent as to adversely affect their lifestyle (Gath et al 1987). Similar findings were observed in other studies (Haynes et al 1977; Chimbira et al 1980; Fraser et al 1984). However, with these data it is apparent that the perception of menorrhagia within a population is variable, but leads to the assumption that a great many women would demand treatment for what they perceive as heavy periods. The demand on resources would also be higher than population studies suggest.

The duration of bleeding has generated less interest. However, the World Health Organisation has a method of assessing the bleeding and spotting patterns (Belsey and Farley 1988). Normality is defined as a bleed of 5 days or less and total bleeding days less than 20 in every 90 (Datey et al 1995).

The actual extent of the problem in the general population is not known, but women presenting to their general practitioners (GPs) with menstrual problems is common (Coulter et al 1991). Approximately 5% of women of reproductive age will consult their GPs each year because of menstrual

abnormalities (Vessey et al 1992; Anonymous 1994). Of all referrals to a hospital gynaecologist for abnormal bleeding, approximately 75% are for (subjective) excessive MBL; indeed one third of all gynaecology referrals are for excessive MBL (Coulter et al 1989; Coulter et al 1991). Once referred they have a high chance of a surgical intervention, mainly because they are reluctant to retry medical treatments or try different medical treatments (Cooper KG et al 1997). Sixty percent of women referred to a gynaecologist with menorrhagia will eventually have a hysterectomy (Coulter et al 1991). Indeed, 44% of women referred with any gynaecological complaint will eventually have a hysterectomy (Coulter et al 1991). Fibroids are the commonest reason for hysterectomy in the UK (38%); but menstrual problems in the absence of fibroids account for 35% of all hysterectomies (Vessey et al 1992). In the Netherlands 14.6% of all hysterectomies were performed in women under 45 years with menorrhagia with no pathological abnormalities.

Hysterectomy remains one of the commonest operations in the western world (Dicker et al 1982; Wingo et al 1985; Loft et al 1991; Coleman et al 1997), with rates increasing. Scottish hysterectomy rates have doubled between the 1960s and 1980s (Anonymous 1995; Roberts et al 1996). In the UK about 20% of women will have had a hysterectomy by the age of 55 years (Vessey et al 1992). The hysterectomy rates in Australia and New Zealand are 25% and 20% respectively for women before the menopause (Coleman et al 1997). In New Zealand 90% of these hysterectomies are performed for heavy menstrual blood loss and fibroids (Coleman et al 1997).

In the past decade, there has been a huge increase in the number of surgical treatments available for women with menorrhagia, without having to resort to hysterectomy (Garry 1997; Garry 2002). The aim of all these techniques is to improve symptoms with minimal morbidity and mortality, reduced hospital stay and a quicker return to normal. However, since the introduction of more of these newer techniques to the UK, there seems to have been little impact on the overall number of hysterectomies performed (Prentice 1999) (table 1.1). There are many reasons put forward for this. The threshold for surgical intervention with minimally invasive (and minimal access) procedures is

	1989-1990	1990-1991	1991-1992	1992-1993	1993-1994
Abdominal hysterectomy	60 554	57 747	58 446	59 607	59 376
Vaginal hysterectomy	12 726	12 928	13 184	13 562	14 141
All hysterectomies	73 280	70 675	71 630	73 169	73 517
Therapeutic hysteroscopy	1 699	4 244	7 707	9 982	9 945
All operative procedures	74 979	74 899	79 377	83 151	83 462

Table 1.1 : Number of operative interventions from 1989-1994 in the UK (from (Prentice A 1999).

undoubtedly lower, both from the patient's and clinician's perspective. Hysterectomy is merely delayed rather than prevented in some cases, as 16% of women undergo hysterectomy within 12 months of a minimal access procedure (Pinion et al 1994).

The majority of these newer minimal access techniques used in the treatment of heavy menstrual loss involve a trans-cervical approach to endometrial

destruction. Some are well-established techniques whilst other newer methods are undergoing assessment. Most appear to offer short-term improvement rates of the order of 80-90 %. They are not without complications and generally require a little skill. They will be discussed in more detail in later chapters.

1.2 The aetiology of heavy menstrual blood loss.

There are population differences in menstrual blood loss. The range of mean blood loss (MBL) in Western Europe is 34.6 ml (in the UK) to 43.4ml (in Sweden) (Hallberg et al 1966; Cole et al 1971; Guillebaud et al 1978). There are few studies outside Europe. One from Egypt showed a MBL of 25.6 ml (Hefnawi et al 1979). One from China showed a MBL of 56.3 ml, with 20% of women having menorrhagia (blood losses of greater than 80ml per month) (Gao et al 1981). Population differences can be explained by either a genetic disposition or an environmental one. Rybo and Hallberg found a greater difference in menstrual blood flow in dizygotic twins than in monozygotic twins (Rybo and Hallberg 1966). Diet undoubtedly plays a part in the control of menstrual blood loss. The composition of the diet can modulate arachidonic acid release in the endometrium and thus modulate prostaglandin-controlled haemostasis in the endometrium. Onions and garlic have been shown to inhibit platelet aggregation and to cause mild bleeding disorders (Makheja et al 1979).

Age may have an effect on the amount of menstrual blood loss (Barer and Fowler 1938; Hallberg et al 1966; Rybo 1966; Janssen et al 1995). Blood loss does tend to be lighter at menarche and heavier at the menopause. The age of menarche has little effect (Rybo 1966). However in some adolescent (Fraser 1985) and perimenopausal women (van Look et al 1977) dysfunctional uterine

bleeding can occur with chronic anovulation. In the majority of women with regular but heavy periods, no hormonal abnormalities can be found (Haynes et al 1977). The impact of parity and infant birth weights on menstrual loss is unclear from the literature (Rybo and Hallberg 1966; Rybo 1966; Cole et al 1971; Gao 1990).

1.2.1 Dysfunctional uterine bleeding.

In the vast majority of cases, menorrhagia is unexplained and known as dysfunctional uterine bleeding (DUB). DUB is best described as abnormal bleeding from the uterine cavity in the absence of organic disease of the genital tract (Davey 1995). There are several theories to explain the blood loss in these women. The main factor is haemostasis in the endometrium

Haemostasis occurs when platelets adhere to the subendothelium of damaged blood vessels, followed by further platelet aggregation, degranulation and fibrin deposition. In the skin this results in the formation of a platelet plug which seals the damaged vessel. However, this normal haemostatic mechanism is inhibited in the endometrium. The platelet plug formation in the endometrium is much smaller, often failing to completely occlude the vessel, and there seems to be little, if any, haemostatic reaction to endothelial damage.

Women with menorrhagia (who have no bleeding disorders) have higher concentrations of certain clotting factors: factor V and VIII, antithrombin III, and alpha 1-antitrypsin (Hahn et al 1975). Levels of albumin caeruloplasmin and IgG are also lower (Hahn et al 1976).

The fibrinolytic activity on the endometrium is higher in the secretory phase of the menstrual cycle than in the proliferative phase. The highest concentrations of plasminogen activators are found in the late secretory phase and during menstruation. The presence of a systemic (Cole and Clarkson 1972; Hahn et al 1976) or a local (Rybo 1966; Hahn and Rybo 1975; Rees et al 1985) fibrinolysis system in women with menorrhagia remains unsolved. The evidence that there is a local mechanism that is at least partly responsible for DUB is growing (Rees et al 1985). The benefits of antifibrinolytic drugs support this hypothesis (Nilsson and Rybo 1971).

1.2.2 Prostaglandins

Prostaglandins have an important role in haemostasis in the endometrium, and in menstruation (Baird et al 1996). Cyclo-oxygenase metabolises arachidonic acid to unstable endoperoxidases (PGG_2 and PGH_2). From these precursors, thromboxane A_2 is formed in platelets, which induces platelet aggregation and degranulation. It is a potent vasoconstrictor. In the endothelium and smooth muscle PGG_2 and PGH_2 are transformed into mainly prostacyclin (PGI_2). PGI_2 inhibits platelet aggregation and degranulation, and is a potent vasodilator. The endoperoxidases can also be transformed into PGD_2 , PGE_2 and PGF_2 . PGD_2 is an inhibitor of platelet function, whilst PGE_2 and PGF_2 are weak stimulators. PGF_2 also acts as a weak vasoconstrictor.

All these prostaglandins are present in the human endometrium. Cyclo-oxygenase is present in the surface and glandular epithelium of luteal phase endometrium. It is not present, however, in the endometrial stroma, the myometrium or the blood vessels (Rees et al 1984; Rees et al 1987; Rees 1990). Changes in endometrial prostaglandin metabolism have an important

role in the regulation of menstruation. This is supported by the fact that prostaglandin inhibitors decrease menstrual blood flow (Anderson et al 1976).

Recently the role of nitric oxide (NO) has been investigated. It is present throughout the uterus and acts as a potent vasodilator and inhibitor of platelet aggregation. It may mediate the vasodilator effects of oestrogen and PGE₂. Disturbances in its function have been implicated as a cause of menorrhagia (Telfer 1997).

1.3 Causes of menorrhagia.

1.3.1 Organic causes.

Local conditions, which can cause menorrhagia, are adenomyosis, pelvic inflammatory disease, endometrial hyperplasia and benign tumours of the cervix or endometrium (polyps and fibroids) or malignant tumours (whether cervical or endometrial) (Fraser et al 1984). Fibroids are found in 25 - 30% of women over the age of 35 years. It is difficult to determine how many women with menorrhagia have fibroids as an aetiological factor. It has been shown that 30% of women undergoing myomectomy suffered from menorrhagia, but the proportion varies from 17 to 62%, (Buttram and Reiter 1981).

Menstrual blood loss in women with endometrial polyps, adenomyosis, pelvic inflammatory disease has been measured by Fraser and co-workers (Fraser et al 1986). They concluded that these conditions were not always associated with menorrhagia.

1.3.2 Intrauterine contraceptive devices.

The insertion of a non-hormonal intrauterine contraceptive device (IUCD) is associated with an increase in measured menstrual blood loss (Guillebaud et al

1976). Ten to twenty percent of women have their IUCD removed within a few months of insertion because of excessive menstrual loss (DTB 2002). This is the major reason for discontinuing the devices. The incidence of anaemia and menorrhagia are four to five times higher in users of IUCD than in women using other forms of contraception (Guttorm 1971). The structure of the endometrium at the time of menstruation in IUCD users shows specific features: the formation of the haemostatic plug is delayed and there is reduced rapidity of endometrial shedding (Christiaens et al 1981). Different devices have different rates of removal for excessive bleeding. Removal rates at 3 years were 4.5% with GyneFix a frameless device; 7.5% with FlexiGard; 8.3% with Cu-Fix and up to 11.5% with TCu 380A (Rosenberg 1996; DTB 2002).

The Mirena intrauterine system (IUS), is a levonorgestrel-containing device. Its structure is very similar to the copper containing IUCDs mentioned above, but users have lighter less painful periods (Tang & Lo 1995). The Mirena IUS is mentioned later in chapter 3.

1.3.3 Tubal ligation.

The incidence of menorrhagia in women after tubal ligation has ranged from 2.5-50% (Rioux 1977). Hysterectomy rates after sterilization by tubal ligation are higher than in unsterilised women (Treloar et al 1999). Some studies did not correct for previous use of the contraceptive pill, nor previous dysfunctional uterine bleeding. Two studies revealed that a third of women had longer and heavier periods following laparoscopic sterilisation (Letchworth and Noble 1977; Jackson and Lander 1980; Goh et al 1981). Women using the oral contraceptive pill before surgery had the heaviest and

longest periods, whilst women using the IUCD had lighter and shorter periods after sterilisation (Chamberlain and Foulkes 1976). A recent study has shown no difference in MBL after tubal ligation, whichever technique is used (Sahwi et al 1989).

Two studies corrected for these factors and found that the incidence of menorrhagia after tubal ligation was 5.4% (Rubenstein et al 1976) and 6% (Stock 1978), with four percent of all women undergoing further surgery for pain or menorrhagia (Stock 1978). The method of sterilisation affects the incidence of menorrhagia. After diathermy division, tubal ligation and vasectomy, the incidence of menorrhagia resulting in hysterectomy was 9%, 3% and 1% respectively (Letchworth and Noble 1977). Women followed up for ten years after tubal ligation have a 13% incidence of hysterectomy for irregular or heavy menstrual blood loss (Muldoon 1972).

1.3.4 Thyroid dysfunction.

Menstrual abnormalities are related to thyroid dysfunction. Menorrhagia is a common abnormality in women with hyperthyroidism, myxoedema and in those after thyroidectomy (Scott and Mussey 1979). Albright coined the term “*metropathia haemorrhagica*” to describe periods of amenorrhoea interrupted randomly with spotting for menstruation in those women with long-standing hypothyroidism (Scott and Mussey 1979). Thyroid hormone replacement effectively restores normal ovulatory cycles in hypothyroid women (Wilansky and Greisman 1989). There is only one case report of a hypothyroid woman where menstrual loss was measured. The pre-treatment loss was almost 500ml and after treatment reduced to less than 80ml (Higham and Shaw 1992).

1.3.5 Clotting abnormalities.

Menorrhagia has long been associated with platelet abnormalities, von Willebrand's disease, clotting factor deficiencies and haemophilia (see table 1.2) (Quick 1966).

Coagulation disorders	Platelet defects
Von Willebrand's	Thrombocytopenia
Haemophilia A carriers	Glanzmann's thrombasthenia
Prothrombin	Aplastic anaemia
Factors V, VII, IX, X, XI	Leukaemia
Deficiencies	Bernard-Soulier syndrome

Table 1.2 Disorders of haemostasis presenting as menorrhagia.

There have been few studies actually measuring blood loss in these conditions, but subjective analysis has shown that some 70-80% of women with thrombocytopenia or thrombopathia and 50% of women with von Willebrand's disease have menorrhagia (Van Eijkeren et al 1989). In addition, coagulation disorders have been found in 29% of adolescents admitted to hospital with acute menorrhagia where no genital tract abnormality was found (Claessens and Cowell 1982). In those women taking anticoagulants, 50% had objectively measured menorrhagia (van Eijkeren et al 1990).

1.4 Methods of measuring blood loss.

Menorrhagia is a purely subjective complaint, based on the patient's assessment of her menstrual blood loss. Hallberg and colleagues measured menstrual blood loss in Sweden, and found that 40% of women with a menstrual loss of greater than 80 ml did not consider them heavy; while 14% of those with a blood loss of less than 20 ml did consider their periods heavy

(Hallberg et al 1966). Several later studies have confirmed these findings; more than half of the women complaining of menorrhagia in a study by Fraser had a blood loss of less than 80 ml per month, while a fifth had a loss of less than 35 ml when measured objectively. A substantial number of the women in this latter group will have failed medical management of their menorrhagia (Fraser et al 1981). Chimbira and others showed that more than a third of women who stated that their periods were light had a blood loss of more than 80 ml (Chimbira et al 1980).

1.4.1 Pictorial methods.

Women use a pictorial chart of the amount of blood staining on their tampons and sanitary towels to score their menstrual blood loss (figure 1.1). There appeared to be good correlation between the menstrual score obtained by this

INSTRUCTIONS		TAMPON								
		1p	2p	3p	4p	5p	6p	7p	8p	9p
1. Draw your upper and the lower ends of your tampon.										
2. Draw your degree of each level of tampon saturation. 1-4 is the pattern on the chart.										
3. To record the amount of blood loss, make a mark in the box opposite the picture which best describes your tampon. Make a mark in the box opposite the picture which best describes your tampon.										
4. When you finish your period, make a mark in the box opposite the picture which best describes your tampon.										
5. If you notice any blood loss on the tampon, or just on the tampon, make a mark in the box opposite the picture which best describes your tampon.										
6. If you experience any bleeding, make a mark in the box opposite the picture which best describes your tampon.										
7. Please do not forget to return your completed chart to the hospital. It will be used to help you, and to help other women, to understand how to manage their periods. It is important to complete the chart.										

TOWEL	1	2	3	4	5	6	7	8
CLOTS								
FLOODING								

Figure 1.1: Pictorial chart for assessment of women's menstrual blood loss.

method and objective menstrual blood loss (Higham et al 1990). However there is a degree of difficulty in standardising the charts based on differing absorbencies in sanitary products currently on the market, and a recent study was unable to validate Higham's work (Reid et al 2000).

1.4.2 Weight measurements.

This technique calculates the blood loss by comparing the weight of sanitary towels and tampons before and after use (Pendergrass et al 1984). Although this technique is simple to perform, it is inaccurate, as the percentage of blood in menstrual fluid varies considerably between individuals (Fraser et al 1985).

1.4.3 Uterine artery Doppler.

If the pulsatility index (PI) of uterine arteries, arcuate arteries, and radial arteries is measured by transvaginal colour Doppler, there is a significant inverse correlation with the amount of menstrual blood loss, suggesting that women with lower uterine flow impedance, bleed more. The association was specific and not explained by uterine size, fibroids or any other potential confounders (Hurskainen et al 1999). This is an interesting finding, and confirmation of the findings are awaited.

1.4.4 Spectrophotometry.

This is the gold standard test for the assessment of menstrual blood loss. The technique involves collecting all sanitary products, extracting all the blood and then measuring the optical density of resultant solution compared with a known aliquot of the patient's blood. The commonest method is the alkaline haematin method (Cheyne and Shepherd 1970). There have been several modifications on this technique (van Eijkeren et al 1986; Vasilenko et al 1988). These methods are extremely time consuming but do obtain the most accurate determinant of menstrual blood loss (Hallberg and Nilsson 1964). All used sanitary wear is bathed in 5% sodium hydroxide to extract the haemoglobin and to convert it to haematin. The resultant solution is then

measured by a spectrophotometer. The extraction and measurement takes approximately 48 hours.

1.4.5 Radiolabelled red blood cells.

The patient is given compatible red blood cells, labelled with radioactive iron (^{59}Fe) or chromium (^{51}Cr). The radioactivity of the blood extracted from all the used sanitary towels and tampons is then compared with that of the patient's venous blood. This technique is invasive, time consuming and does not have the same accuracy of other methods (Baldwin et al 1961).

Method	Simple for investigator	Simple for woman	Accurate	Patient satisfaction
Pictorial	Yes	Yes	Yes / no	high
Weight	Relatively	Relatively	poor	?
Doppler	Relatively	Relatively	?	?
Spectral	No	No	Gold standard	?
Radio-labelling	No	Invasive	Yes/no	?
Subjective assessment	Yes	Yes	Very poor	high

Figure 1.3: Comparison of different methods of assessing menstrual blood loss.

1.5 Conclusion.

The woman's subjective assessment of her blood loss is the most important factor in the management of menorrhagia. Accurately measuring blood loss is interesting, but does little to help the woman, or her clinician, in treating her complaint. Different methods of assessing blood loss are outlined in table 1.3.

CHAPTER 2: THE ENDOMETRIUM.

2.1 Introduction.

Between the years of the menarche and the menopause, the endometrium of the uterus undergoes monthly cyclical changes in its structure under the influence of ovarian hormonal stimuli. This cycle is naturally only suspended because of pregnancy. Normal menstruation lasts 3 to 5 days, with a cycle of 24 to 32 days, being most regular between the ages of 20 and 40 years. At the menarche and the menopause the cycles tend to be abnormal. The vaginal, cervical and tubal epithelium also shows cyclical response to sex hormones, but not as pronounced as that shown by the endometrium.

The endometrium undergoes changes in both secretory activity and structure, correlated with cyclical growth and maturation of ovarian follicles. The end of each cycle is characterised by the partial destruction and softening of the endometrium. Bleeding from blood vessels within the mucosa assists this.

The endometrium contains a unique system of blood vessels, which undergo developmental changes during the menstrual cycle, in preparation for support of a fertilised ovum.

Branches of uterine arteries pass through the myometrium to supply the endometrium. These divide into two different arteries: the spiral arteries or arterioles which supply the superficial two-thirds of the endometrium; the basilar arteries supply the deeper one-third. The spiral arterioles are highly sensitive to the hormonal changes of the menstrual cycle.

The endometrium is divided into three histologically and functionally distinct layers. At the end of menstruation (menstrual phase) the endometrium consists of a thin band of connective tissue approximately 1 mm thick which contains the basal portions of uterine glands and lower portions of the spiral arterioles. This is the stratum basalis, lying adjacent to the myometrium. This layer undergoes minimal changes during the menstrual cycle. It contains the cells for cyclical regeneration of the upper layers. The broad intermediate layer is called the stratum spongiosum. The thinner superficial layer is known as the stratum compactum. The two superficial layers are jointly referred to as the stratum functionalis.

2.2 Endocrine basis of menstruation.

After ovulation the corpus luteum continues producing progesterone and oestradiol, for 10 days. If fertilisation does not occur, the levels of these hormones decline, as the corpus luteum regresses. Withdrawal of the influence of these hormones on the endometrium will result in menstrual bleeding. The most important hormone is progesterone. Bleeding may occur with withdrawal of oestradiol, but always occurs with progesterone withdrawal, and indeed maintaining levels of progesterone will prevent bleeding.

The majority of women presenting to gynaecological clinics with heavy menstrual blood loss have regular ovulatory cycles (Cameron 1989). Measuring oestradiol and progesterone in these same women with excessive menstrual blood loss has failed to demonstrate abnormalities in their hormonal status (Haynes et al 1977). In order to understand abnormal menstruation, therefore, we must understand normal menstruation.

2.3 Normal menstruation.

In the first few days after menstruation, under the influence of oestrogen the cells in the stratum basalis proliferate. The epithelial cells rapidly cover the denuded endometrial surface. It is lined with simple columnar epithelium, with a mixture of ciliated cells and secretory cells. The stroma cells proliferate, and the spiral arterioles lengthen. This is known as the proliferative phase. This phase continues until the day after ovulation (which in a regular 28-day cycle is approximately on day 14). At the end of this phase the endometrial glands are narrow and straight, and the spiral arterioles are only slightly coiled and do not extend into the upper third of the endometrium.

Under the influence of progesterone produced by the corpus luteum, the endometrium becomes oedematous and thickens to 10 to 15 mm thick. The glands enlarge becoming tortuous, secreting a mucoid fluid, rich in nutrients, in particular glycogen. The spiral arterioles lengthen becoming more coiled, extending nearly to the surface of the endometrium. This is known as the secretory phase lasting to menstruation. The corpus luteum remains active producing oestrogen and progesterone for ten days after ovulation. If fertilisation does not occur hormone levels rapidly decline. The reduction of progesterone at the end of cycle causes the walls of the spiral arterioles to constrict leading to ischaemia in the stratum functionalis. Markee transplanted endometrium into the anterior chamber of the eye of rhesus monkeys, and demonstrated that before menstruation occurs there is intense vasoconstriction of these spiral arterioles. He also noted the increased coiling of the spiral arterioles, as well as a degree of endometrial regression (Markee 1940). As the

spiral arterioles vasoconstrict there is endometrial thinning, which further increases ischaemia. Between 4 and 24 hours after this initial constriction, the spiral arterioles dilate, blood passes into the ischaemic endometrium, and desquamation occurs. Blood, uterine fluid, soft necrotic tissue, (from the stratum functionalis) is therefore lost at menstruation. After 4 to 5 days of bleeding, when only the strata basalis remains, the blood flow stops.

This menstrual blood loss is 75% arterial and 25% venous. Blood clotting in the spiral arterioles is inhibited during the initial blood flow by locally produced prostaglandins. Blood flow to the superficial layers (stratum functionalis) is solely by the spiral arterioles; the deep stratum basalis is served by basilar arterioles unaffected by this process.

Oestrogen and progesterone act via specific endometrial receptors. Two receptor subtypes have been identified for both hormones. The number of the specific receptors ($ER\alpha$ or $ER\beta$) vary in the endometrium throughout the cycle. The predominant form is $ER\alpha$; the expression of which reaches a nadir at the time of menstruation (Salamonsen et al 1999). The progesterone receptors are known as PRA and PRB. PRA is present in glandular and stromal nuclei during the proliferative phase but in only the stromal cells at the end of the secretory phase (Wang et al 1998). PRB is present in both glandular and stromal nuclei during the proliferative phase, but totally absent in the secretory phase (Wang et al 1999). The question that has not been answered is what effect these hormones have - whether a direct effect on the endothelial cells, or whether it is a cytokine-mediated effect.

2.4 Control of Blood Loss - Haemostatic Mechanisms.

2.4.1 Platelet plug formation.

Menstrual fluid can be kept for several weeks without clotting (Schinkele 1912). It is free of platelets and fibrin (Salamonsen et al 1999). Fibrin has been demonstrated in the haemostatic plugs of the endometrium, but it is very rapidly removed (Salamonsen et al 1999). Indeed increased fibrinolysis is associated with the onset of menstruation in vivo (Casslen and Astedt 1983; Glesson 1994a).

Tissue Factor (TF) generates thrombin, converting fibrinogen to fibrin. It is the primary initiator of haemostasis, and is localised within stromal cells within the secretory endometrium (Salamonsen et al 1999). TF levels are increased under the control of progesterone and decreased with its removal (Lockwood et al 1993).

Unusually in the body, the endometrium appears resistant to scar formation. After menstruation the two raw basal layers remain in apposition for a number of days, yet the body's normal healing mechanisms cause no adhesions to form. This is true after parturition as well as menstruation. The coagulation cascade occurs in the endometrium just as in other tissues. Platelets accumulate, degranulate and cause the deposition of fibrin sealing the endometrial vessels, within hours of the onset of menstruation. In order to prevent the formation of a scar and therefore obliteration of the uterine cavity, it is important to prevent clot formation. This is done by two main fibrinolytic mechanisms. Tissue plasminogen activator, and urokinase plasminogen activator initiate fibrinolysis. They are both released by the degrading

endometrium. Oestradiol stimulates and progesterone inhibits urokinase plasminogen activator (Casslen and Astedt 1983; Casslen et al 1986; Casslen et al 1995). Progesterone also inhibits tissue plasminogen activator. Thus as progesterone levels fall causing the onset of menstruation, and oestradiol levels rise, plasminogen activator levels rise and therefore intrauterine adhesions are prevented.

One of the few situations in which the endometrium fails to regenerate properly was first described by Asherman in 1948: the syndrome of '*amenorrhoea traumatica*'. This amenorrhoea was described either postpartum, or post-abortion curettage. The endometrium in these cases appears insensitive to hormonal stimulation, and intrauterine adhesions form. It has been suggested that vigorous curettage causes adhesion formation, in cases of missed abortion, although it may be due to infection (Asherman 1948, Moyer 1968). These adhesions result from repair by fibrosis instead of endometrial regeneration. It is not understood why this should occur.

Other factors are produced by the endometrium, which inhibit scar formation, namely prostacyclin, nitric oxide and platelet activating factor (Alecozay et al 1991, Kelly et al 1984). No derangement in the levels of these factors or in fibrinolysis has been noted in women with menorrhagia. However, plasminogen activator is important in menorrhagia. Women, who use a copper-containing intrauterine contraceptive device, tend to have heavier periods. The levels of plasminogen activator are increased in these women (Shaw et al 1983). Those women using the combined oral contraceptive pill, which can be used in the management of menorrhagia, have lower levels of plasminogen activator (Casslen and Astedt 1983).

2.4.2 Vasoconstriction.

During menstruation, the platelet-fibrin plug seals the damaged blood vessels. With the loss of further endometrium, these plugs are lost. The blood loss is restricted by the vasoconstriction of the spiral arterioles in the stratum basalis (Christiaens et al 1982). The status of the spiral arterioles' lumen is under the control of the vasoconstricting prostaglandin $\text{PGF}_{2\alpha}$ and the vasodilating PGE_2 . During the luteal phase of the cycle, both are increased, under the influence of progesterone. It has been shown that an increased $\text{PGE}_2 / \text{PGF}_{2\alpha}$ ratio is present in those women suffering from menorrhagia. In these women there is an increased PGE_2 receptor concentration in the endometrium (Adelantado 1988a; Adelantado 1988b; Cameron 1989; Cameron et al 1987; Smith et al 1981). Prostaglandins are produced from arachidonic acid by the action of cyclo-oxygenase. The activity of the cyclo-oxygenase-2 (COX-2) enzyme is induced and regulated by progesterone. COX-2 inhibitors reduce menstrual blood flow. The precise link between prostaglandins and menstruation is not known (Baird et al 1996).

Another extremely potent family of vasoconstrictors are the endothelins, which are found in a variety of tissues including the uterus (O'Reilly et al 1992). There are three endothelin peptides (ET-1, -2 and -3) that are stored in the endometrial glands, and as the endometrium breaks down, are released. There are two specific receptors ETA and ETB. ET-1 is the body's most powerful vasoconstrictor. They cause long-lasting vasoconstriction in the spiral arterioles (Marsh et al 1997). There is evidence that endothelins may be the pressor agents responsible for the intense vasoconstriction seen in the

spiral arterioles at the start of the process of menstruation (Abberton et al 1999).

2.5 Endometrial repair.

Endometrial repair commences under the influence of rising levels of oestrogen, released by the developing follicle. This repair is mediated by epidermal growth factor (EGF), produced by the denuded secretory spongiosum (Ishihara et al 1990). EGF contributes to both glandular and stromal repair (Haining et al 1991). Oestrogen also stimulates the production of vascular endothelial growth factor (VEGF) (Charnock-Jones et al 1993). Endometrial repair lasts 48 hours (Ferenczy 1976). This replaces the surface epithelium and is derived from the exposed end of basal glands, and persistent and intact epithelium within the cornual and isthmic regions of the uterus.

By day 5 the surface epithelium is intact. There is then a rapid change in the endometrial components (Ludwig et al 1990). Loss of the functional layer is responsible for stimulation of endometrial repair, and the rapid mitosis and ciliogenesis only occur after this is complete. If the superficial layers of epithelium are not totally lost during menstruation, there will be no nude basalis. This might in part be responsible for heavy or prolonged menstrual blood loss. Therefore if this overlying tissue is removed from the basal layer, as during a dilatation and curettage, epithelial regeneration can occur and bleeding will stop (Ludwig et al 1990).

Very little is known of the molecular and cellular basis of endometrial repair. Numerous growth factors have been implicated and a great deal of work is ongoing. Better treatment modalities for the treatment of menorrhagia may

result from improved knowledge of the process of endometrial regrowth after menstruation.

Various techniques have been employed, over the years, to stop women with excessive menstrual blood loss from bleeding. Apart from hysterectomy, surgical methods currently used aim to prevent endometrial re-growth, and therefore to prevent further menstrual blood loss, by destroying or removing the endometrium leaving no glandular elements for subsequent re-growth. This is discussed later.

2.6 Methods of assessment of the endometrium and uterine cavity.

Abnormal uterine bleeding after the age of 40 years requires further evaluation to exclude intrauterine pathology (RCOG 1994; RCOG 1998). Patients under 40 years old have a very little risk of developing endometrial cancer (Mackenzie and Bibby 1978). However, the endometrial cavity and endometrium should be assessed in the younger woman if abnormal bleeding persists after a trial of medical treatment, or where there are risk factors such as obesity and diabetes.

2.6.1 Ultrasound.

Ultrasound can be used to assess the endometrium, the myometrium and adnexae. Transvaginal ultrasound scanning (TVS) uses higher frequency sound waves than transabdominal scanning, giving better quality images at the expense of decreased depth of penetration. The RCOG Guideline Development Group has summarised the evidence. It states that TVS is

superior to transabdominal scanning at visualising the uterine cavity (RCOG 1999).

There have been few studies examining the accuracy of ultrasound. Two small studies demonstrated that endometrial thickness measured by ultrasound agreed with histological assessment (Fleischer et al 1986; Saxton et al 1990). Echoes of high intensity usually imply the presence of endometrial abnormalities (Weigel et al 1995). Endometrial cancer is suspected when endometrial thickness is 20 mm or more (Smith-Bidman et al 1998), together with the presence of hypo-echoic areas, and a heterogeneous appearance (Sheth 1993). Endometrial polyps may appear as cystic spaces and all the endometrium may appear hyperechoic (Hulka 1994; Kupfer 1994). Endometrial thickness (ET) is used to detect intrauterine pathology. Atrophic endometrium is 3mm thick or less. In women with post-menopausal bleeding a cut off thickness of 5mm has been shown to have a sensitivity of 100% with a specificity of 96% of identifying endometrial cancer (Granberg et al 1991). Some cancers have been missed at this level (Bombieri et al 1996). A bigger study has suggested a cut-off of 4mm which detected 100% of all cancers (Karlsson et al 1995). This level does not detect all pathologies. 5.5% of benign pathologies are missed, but this was thought to be acceptable by the authors who cited a 6% failure rate for D&C (Karlsson et al 1995).

Ultrasound detection of endometrial polyps is, however, unreliable (Fleischer 1986, Fleischer et al 1987), due to the normal range of endometrial thickness overlapping with that seen in women with polyps (Dijkuizen 1986). Detection of polyps by TVS varies between 90% (Indman 1995), and 58% (Cacciatore

1994). TVS has been reported to detect almost 100% of submucous fibroids (Indman 1995; Fedele 1991).

Colour flow doppler scanning may assist the diagnosis of endometrial carcinoma due to the increased blood flow in malignancy (Kurjak 1993; Campbell et al 1993), but increased blood flow has also been reported in benign conditions (Kupesic-Urek 1993).

Saline sonography is when saline is introduced into the uterine cavity through a catheter under ultrasound observation. It allows better detection of polyps, submucous fibroids and other pathologies, and can distinguish between these pathologies (Syrop et al 1992; Goldstein et al 1990; Bourne et al 1994). Detection rates for intrauterine pathologies are increased from 23.5% with transvaginal ultrasonography to 94.1% with saline sonography (Krampl et al 2001). However, there is a possible risk using saline sonography, of intrauterine infection and spread of malignant cells into the pelvic cavity. There is an element of discomfort associated with this.

TVS has been found to be a good, non-invasive method of endometrial assessment when compared with hysteroscopy and curettage. It has a sensitivity and specificity of 96% and 89% respectively for identifying endometrial pathology (Emanuel et al 1995).

2.6.2 Magnetic resonance imaging (MRI).

MRI can be used to obtain measurements of endometrial thickness, diagnose intrauterine pathology and identify structural abnormalities of the uterus (Dudiak et al 1988; Ascher 1996). Its value however must be tempered



Fig 2.1: MRI sagittal section through pelvis showing posterior fibroid and menstrual blood within the cavity

against the high costs and the timescale involved in obtaining an image, compared with other methods of assessing the endometrium (figure 2.1).

2.6.3 Dilatation and curettage.

This procedure was introduced by Recamier in 1843 (Ricci 1949) and until recently had been considered the standard investigation for endometrial assessment. Certainly the annual rate of this procedure at the end of the 1980s was 71.1/10,000 in England (Coulter et al 1991). Dilatation and curettage (D and C) has limitations and complications and has been largely replaced with hysteroscopy. It is a blind procedure where in the majority of cases less than half of the endometrial cavity is sampled, and whereby small polyps and sub

mucus fibroids may well be missed. Uterine perforation occurs in approximately 1% of cases. Haemorrhage rates of 0.4% and infection rates of approximately the same occur. Diagnostic curettage may rarely also give rise to intrauterine adhesions (Shearman 1995). Cervical damage may also occur as a result of forced dilatation of the cervix. Generally this is a procedure that is performed under a general anaesthetic.

2.6.4 Hysteroscopy.

The first hysteroscopy was performed in 1869, when polyps were diagnosed in a woman with post-menopausal bleeding (Lindemann 1973). With the advance in technology, hysteroscopy is now offered as an outpatient 'office' procedure performed under local anaesthetic, whereby the whole surface of the endometrium can be directly visualised (Cicinelli 1994). It allows the possibility of directed biopsy of specific areas of the endometrium. Carbon dioxide (CO₂), saline or glycine are generally used to distend the cavity. Carbon dioxide is used rather than air because of a decreased risk of gas embolism (Weismann 1996). Hysteroscopy can detect small polyps, submucous fibroids and malignancies which would have otherwise have been missed by outpatient biopsy, ultrasound or D & C (Gimpleson 1988; Loffer 1988; Towbin et al 1996). The use of pelvic ultrasound can decrease the need for invasive diagnostic hysteroscopy (Jones and Bourne 2001). However detection rates for intrauterine pathology using transvaginal ultrasound have been quoted as low as 23.5%, although using saline sonography can significantly increase this to over 94% (Krampl et al 2001). Hysteroscopes are becoming smaller, the optical systems are getting better, and now with 2mm

diameter flexible hysteroscopes, the need for dilatation of the cervix, and therefore local anaesthetic is less (Bradley 1995). Hysteroscopy should not be carried out in a pregnant woman, or in women with known acute pelvic inflammatory disease (PID), or known endometrial cancer (Lewis 1990) for fear of disease spread. In women with a history of PID, or ectopic pregnancy, antibiotic prophylaxis should be used for Chlamydial infection.

2.6.5 Endometrial biopsy in the outpatient setting.

Most women in the outpatient setting will tolerate an endometrial biopsy. They are simple and safe to perform. There is no need for a general anaesthetic, and the biopsy devices are relatively inexpensive. The two most commonly used devices are the Pipelle (Unimar, Connecticut, USA), and the Vabra aspiration biopsy (Berkley Medevices, Berkley, California, USA).

The Pipelle sampler is a 3.1 mm diameter flexible plastic cannula 23.5 cm long. There is a distal aperture in the side of the cannula through which the endometrial specimen is aspirated. The device is inserted through the cervix; the length of the cavity is measured with centimetre depth markings. Once inserted a piston within the device is withdrawn to create a vacuum and the whole device is rotated while being withdrawn. An adequate endometrial specimen is obtained in 90% of women (Forthergill et al 1992; Kaunitz et al 1988). There is a 98% detection rate for endometrial cancer using Pipelle (Stovall et al 1991). However another study has disputed this (Ferry 1993) with only a 67% detection rate. Pipelle only samples 4% of the endometrium (Rodrigues 1993).

The Vabra curette is a stainless steel cannula 24 cm long and 3 mm in diameter. It has a chamber for collection of the specimen at one end, connected to an electric vacuum pump. The device is inserted into the cavity, rotated and withdrawn. The Vabra can detect 95% of malignant intrauterine pathology (Grimes 1982). Vabra samples 42% of the endometrium (Rodrigues 1993).

Other endometrial biopsy devices (e.g. Explora, Accurette, Z-sampler) have similar detection rates and sampling rates (Spencer 1999). However, all these devices are 'blind' samplers. Not all the endometrial surface will be sampled, and polyps or other pathology may be missed.

CHAPTER 3: TREATMENT OPTIONS OF MENORRHAGIA.

3.1 Introduction.

Five per cent of women aged 25 to 44 years will consult the GP about excessive menstrual loss every year (Vessey et al 1992). More than one quarter of the female population consider menstruation excessive (Gath et al 1987). In fact 22% of women in this study stated that their menstrual loss was sufficient to adversely affect their lifestyle. Nearly 10% of employed women take time off work because of excessive menstrual loss (Gath et al 1987).

Treatment options may be medical or surgical. The traditional surgical treatment is a hysterectomy. This has a cure rate of 100%, and is the treatment of heavy menstruation by which all other treatment modalities should be assessed. Sixty percent of women referred to hospital with menorrhagia will have a hysterectomy within 5 years (Coulter 1991). There are risks associated with hysterectomy, and there is a high morbidity associated with the operation which will be discussed later in this chapter. Other more minimal surgical techniques will be discussed which improve morbidity. Surgical treatments have a major disadvantage in that they are not suitable for women who have not completed their family.

Several medical treatments are available which women wishing children, or those who do not wish a surgical option can use. The ideal treatment option for menorrhagia should be cheap, easy to administer, effective and have no side effects. Only 58% of women are offered medical treatment by their GPs prior to referral to a gynaecologist in the UK (RCOG 1998).

3.2 Medical treatments.

3.2.1 Anti-fibrinolytics.

Tranexamic acid acts by inhibiting tissue plasminogen activator, a fibrinolytic enzyme found to be raised in women with dysfunctional uterine bleeding (DUB). It has been shown to reduce menstrual blood loss by just over 50% (Nilsson and Rybo 1971; Andersch et al 1988; Milsom et al 1991).

The most common side effect of tranexamic acid are nausea and diarrhoea. There have been reports of an increase in arterial thrombosis associated with its use (Agnelli et al 1982). However, tranexamic acid has been used for many years in Scandinavia, and analysis of 19 years worth of data showed that the incidence of thromboembolic events was the same as the general population (Lindoff et al 1993). Preston and co-workers studied 46 women with menorrhagia and allocated women to Tranexamic acid or norethisterone (Preston et al 1995). They found a reduction in mean menstrual blood loss of 45% with Tranexamic acid, but a 20% increase with norethisterone. Another study comparing Tranexamic acid with mefenamic acid and ethamsylate showed a reduction of 58% in menstrual blood loss with Tranexamic acid, a 25% reduction with mefenamic acid, and no change with ethamsylate (Bonnar and Sheppard 1996).

3.2.2 Non-steroidal anti-inflammatory drugs.

The non-steroidal anti-inflammatory drugs (NSAIDs), include aspirin, ibuprofen, naproxen and mefenamic acid. These drugs act by inhibiting cyclo-oxygenase, and therefore reducing the production of endoperoxidases and

endometrial prostaglandins. The fenamates (mefenamic acid) also block myometrial PGE₂ receptors.

There are many randomised trials of mefenamic acid used in menorrhagia. Reductions in blood flow of between 20% (Dockeray et al 1989) and 47% (Hall et al 1987) are quoted. It appears that women with the most severe menorrhagia benefit the most. Other NSAIDs have not been studied to such an extent. Hall et al found naproxen to be as effective as mefenamic acid in a crossover trial (Hall et al 1987). Ibuprofen reduced MBL by 13% (Makarainen and Ylikorkala 1986).

The NSAIDs are effective, well tested and well tolerated drugs side-effects are outlined in table 3.1).

3.2.3 Ethamsylate.

The mechanism action of ethamsylate is not well understood. It is thought to decrease capillary fragility, and also has effects on prostaglandin synthesis. Two small studies have shown it is effective in reducing MBL by 50% in women with menorrhagia (Harrison and Cambell 1976; Chamberlain et al 1991). This study used ethamsylate for a total of 15 days per cycle. Other studies restricting treatment to the days of the menses have been less successful. There was only a 7% reduction in IUCD users (Kasonde and Bonnar 1975). In a randomised controlled study comparing ethamsylate with mefenamic acid and tranexamic acid, there was no reduction in menses with the ethamsylate (Bonnar and Sheppard 1996). Ethamsylate is little used in the UK and because of commercial pressures the IV preparation was withdrawn from use in the UK in 2000.

Drug	Maximum reduction in MBL (%)	Side effects
Antifibrinolytics (Tranexamic acid)	50%	Nausea, vomiting, diarrhoea, colour vision disturbances
COCP	50%	Headaches, migraine, weight gain, nausea, breast tenderness, hypertension, thromboembolic events
Progestogens	20%	Weight gain, nausea, bloating, oedema, headaches, acne, depression, exacerbation of epilepsy and migraine, loss of libido
NSAIDs (Mefenamic acid)	50%	Nausea, vomiting, gastric upset, diarrhoea, headache, bronchospasm, thrombocytopenia
Danazol	98%	Weight gain, headaches, acne, depression, loss of libido, rash, hirsutism, voice changes, diminished breast size, mood changes
Ethamsylate	50%	Nausea, headache, rashes
GnRHa (Buserelin Goserelin)	95%	Hot flushes, night sweats, loss of libido, dry vagina, lethargy, headaches, osteoporosis.

Table 3.1: Potential side effects with medical treatments

3.2.4 Cyclical Hormonal treatments

3.2.4.1 Progestogens.

Norethisterone is one of the most widely used treatments for menorrhagia, it has been used for over 30 years (Bishop and de Almeida 1960). It is probably the least effective of all treatment modalities. The change in blood loss in one

study was of an increase of 20% (Preston et al 1995). However, the use of progestogens whether as norethisterone or medroxyprogesterone acetate support from day 5-26 in irregular non-ovulatory cycles, reduced blood loss by 51% (Fraser 1990). Norethisterone has been shown to be of benefit in the minority of women with anovulatory menorrhagia, most especially when the treatment is extended to 21 days (Fraser 1990). Side-effects are dose-dependant and are outlined in table 3.1.

3.2.4.2 *The combined oral contraceptive pill (COCP).*

Nilsson and Rybo (Nilsson and Rybo 1971) used various contraceptive pills and found a mean decrease in blood loss of 53%. An added benefit of the COCP is that it can regulate cycles. The quantity of hormones in the preparations of almost 30 years ago were much greater than today, but studies are awaited investigating menstrual blood loss and the newer COCP. The COCP, however, is restricted in its use in older women and those who smoke, and those with risk factors for thrombosis. Despite the documented reduction in MBL, only 11% of GPs prescribe it for treatment of menorrhagia (Coulter et al 1995). A review of trials of oral contraceptive pills for menorrhagia was unable to reach conclusions because of the paucity of adequate data (Cochrane Database 2000).

Part of the reason why the COCP is not prescribed more frequently despite its success in the treatment of menorrhagia is the fear of thromboembolic disease, especially in the older age group. In the absence of additional risk factors such as smoking, previous thromboembolic episode or family history, the COCP is not contra-indicated. However, third-generation COCP have been associated

with an increased risk of thromboembolic disease (Jick et al 1995; Poulter et al 1995) and this should be borne in mind when prescribing.

The mode of action in menorrhagia is by suppression of the endometrium, reduction of endometrial prostaglandin synthesis and altered fibrinolysis within the uterus (Irvine and Cameron 1999).

3.2.5 Continuous Hormone Treatment.

3.2.5.1 *Danazol.*

Danazol, a synthetic steroid, inhibits sex steroid synthesis, blocks androgen and progesterone receptors, and inhibits pituitary gonadotrophins at higher doses. It thereby inhibits endometrial growth. Its use is limited because of androgenic side effects.

It was first used in the treatment of menorrhagia in 1979 (Chimbira et al 1979). Three large studies of danazol use have shown significant reduction in blood flow. One study used 400 mg, 200 mg, and 100 mg with reductions of 98%, 86%, and 57% respectively (Chimbira et al 1980). Another used 100 mg comparing it with mefenamic acid. This study found a 60% decrease in blood loss with danazol compared to 20% with mefenamic acid, however three quarters of women had side-effects with 40% stopping treatment because of this (Dockeray et al 1989). Higham and Shaw compared danazol with norethisterone 5 mg twice daily (Higham and Shaw 1993). Continuous 200 mg of danazol reduced blood flow by 39%, whereas there was a mean increase of 9% in the norethisterone group.

3.2.5.2 *Gestrinone.*

This is a synthetic derivative of 19-nortestosterone. It has both anti-oestrogenic and antiprogesterogenic properties. More than 50% of women will be made amenorrhoeic and a further 25% will have significant reduction in MBL (Chimbira et al 1980).

3.2.5.3 *Gonadotrophin-releasing hormone analogues.*

The gonadotrophin-releasing hormone analogues (GnRHa) can be used continuously. There is pituitary down-regulation and inhibition of the production of luteinising hormone and follicle stimulating hormone. The hypo-oestrogenic state that they produce is associated with menopause-like side effects - such as hot flushes and dry vagina. They are effective in producing amenorrhoea (Thomas et al 1991). Their use is limited to six months because of the risk of osteoporosis (Dawood et al 1989). Bone loss over this period is less than 5%, and is 8% over a twelve-month period; but there is a question as to whether this is reversible (Fogelman 1996; West et al 1987). GnRHa may be given as a nasal spray several times a day (bruserelin and nafarelin); as a subcutaneous injection monthly (leuprorelin and goserelin) or as a monthly intramuscular injection (leuprorelin).

3.2.5.4 *Tamoxifen.*

This is an anti-oestrogen with weak oestrogenic activity, tamoxifen has not been used widely in the treatment of menorrhagia. However, when used it does reduce menstrual blood loss, but with a risk of endometrial polyps (Fraser 1987).

3.2.6 Local Progestogen Treatment.

3.2.6.1 *Levonorgestrel Intrauterine System (LNG-IUS).*

The levonorgestrel intrauterine system (LNG-IUS) (Mirena®; Sherring, UK), is licensed in the UK for contraception and in the treatment of menorrhagia. It consists of a plastic T-shaped frame with a steroid reservoir containing 52 mg of levonorgestrel. Levonorgestrel is the isomer of norgestrel, a derivative of the 19 nor-testosterone progestones. It is released as a rate of 20µg per day. This release is sustained over seven years (Hollingworth & Guillebaud 1994).

The effect on the endometrium is mediated via a decrease in oestrogen receptors and an increase in the 17OH-oxoreductase activity, which converts oestradiol to oestrone. Progestogens inhibit mitotic activity; the LNG-IUS has been shown to be effective at controlling endometrial hypertrophy by suppressing endometrial growth. After only a few weeks there is glandular atrophy, mucosal thinning and the epithelium becomes inactive. There is also suppression of the spiral arteriolar growth. The endometrium becomes unresponsive to oestrogen with reduced (if any) shedding, due to down-regulation of the oestrogen receptors (Luukkainen and Toivonen 1995; Luukkainen et al 1990). These endometrial changes are uniform within three cycles after insertion (Luukkainen and Toivonen 1995), and after the system is removed, the morphology of the endometrium returns to normal, including normal menses within 30 days (Nilsson et al 1977). The majority of women continue to have normal ovulatory cycles (Luukkainen et al 1990). Uterine bleeding has no effect on ovulation. It has been shown that there were no differences in oestradiol and progesterone levels between users with amenorrhoea or those that were continuing to menstruate (Nilsson et al 1984).

The LNG-IUS has been shown to be highly effective at reducing menstrual blood flow in pre-menopausal women with menorrhagia (RCOG 1998; Tang & Lo 1995; Irvine et al 1998). After three months use there was an 85% reduction in menstrual blood loss, the number rising to 97% after 12 months, with 35% of women amenorrhoeic at one year (Andersson and Rybo 1990; Tang & Lo 1995; Irvine et al 1998). In a study of 50 women who were awaiting a hysterectomy or endometrial resection for menorrhagia, 82% who were fitted with a LNG-IUS were satisfied with their menstrual loss by six months and 8% had amenorrhoea. Fifty-six percent of the women noticed an improvement or cure in premenstrual syndrome symptoms and 80% had a reduction in dysmenorrhoea. Of interest was the fact that 82% of these women asked to be taken off the waiting list, as the treatment was so successful (Barrington and Bowen-Simpkins 1997). In a RCT, women on the waiting list for hysterectomy were offered an LNG-IUS fitted 6 months prior to their operation or to continue their medical treatment. Sixty-four percent of women in the LNG-IUS group and 14% in the medical group removed themselves from the list as they were so satisfied (Lahteenmaki et al 1998).

As the endometrium takes about three months to atrophy, bleeding within this time can be highly erratic, but generally settles by 6 months. Women may suffer from regular spotting in the first 3-6 months of use (Datey et al 1995).

Despite the fact that serum levels of levonorgestrel are very low, some women seem to have adverse progestogenic side effects associated with the use of the LNG-IUS. These may be physical, such as oedema, headache, breast tenderness and even hirsutism and acne. There is also evidence of decreased

LDL levels (Sturridge and Guillebaud 1996). The physical effects are not persistent.

The counselling of women about spotting is important, yet women must also be counselled about the reduction in menstrual blood flow and the possibility of complete cessation of menses, and that this is not a pathological situation. It is important to stress that despite cessation of menses, ovarian function will remain normal. Otherwise women may request the IUS is removed unnecessarily. In the largest study of removal of the LNG-IUS, involving 17,360 users in Finland, the one, two, three, four and five year continuation rates were 93%, 87%, 81%, 75% and 65% respectively, with spotting and excessive bleeding being the primary reasons for removal (Backman et al 2000).

Insertion of a LNG-IUS prior to endometrial ablation can remove the need in approximately 75% of cases (Romer 2000). The 1993 costs of 822,000 prescriptions for 345,255 women was £7 million (NCRD 1995)

3.3 Surgical management.

3.3.1 Hysterectomy.

Soranus of Ephesus performed the first hysterectomy vaginally over 17 centuries ago (Ewen and Sutton 1995). Numerous other reports occurred in the 16th and 17th century. Reports of the first total abdominal hysterectomy (TAH) of the modern age occurred in Manchester in the UK in May 1844, by Charles Clay. The patient died of torrential haemorrhage. In 1850 in Massachusetts, Ellis Burnham performed the first TAH where the patient survived. However the mortality rate from his operation remained around

80%. Overall mortality from TAH still exceeded 70% in 1880, but by 1930 the mortality rate was less than 3% (Ewen and Sutton 1995).

With the further improvement in anaesthesia and antibiotics, mortality is now less than 1 per 1000 cases, but the morbidity is high, with almost 50% of patients suffering from one or more complication (Dicker et al 1982). In 1985

(Ritch 1999). These laparoscopic hysterectomy (LH) techniques involve hysterectomy without a large abdominal wound. They can vary from a

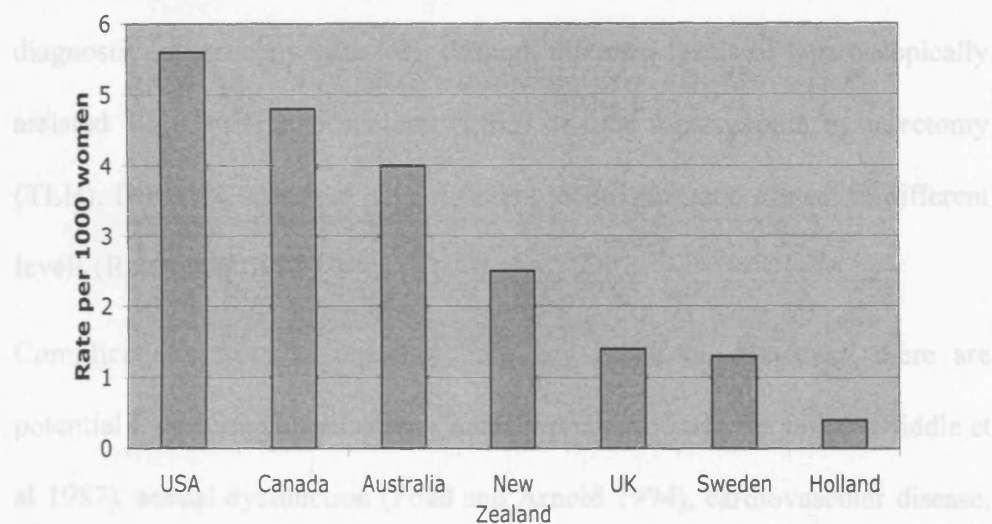


Figure 3.1: Hysterectomy rates from around the world (from Hill 1997).

there were over 60,000 hysterectomies performed in the UK (Last 1989), although the rate in the UK is below the rate in other countries (figure 3.1). In 2000-2001 there were just over 47,000 (NICE 2004), the difference being accounted by the increase in MAS techniques and the Mirena IUS.

The mortality rate for hysterectomy, excluding cancer and pregnancy is 6 per 10,000 (Wingo et al 1985). The Confidential Enquiry into Perioperative

Deaths in 1996-7 reported 7 deaths due to haemorrhage and / or infection in women having a hysterectomy for benign disease (Anonymous 1998).

Some surgeons believe that all hysterectomies should be done vaginally (Moen et al 1995). There are several techniques of performing a vaginal hysterectomy (VH). Other surgeons are very pro-laparoscopic techniques (Reich 1999). These laparoscopic hysterectomy (LH) techniques involve hysterectomy without a large abdominal wound. They can vary from a diagnostic laparoscopy with VH, through different levels of laparoscopically assisted VH (LAVH) to subtotal (LSH) or total laparoscopic hysterectomy (TLH). Different operators have different techniques, and dissect to different levels (Reich et al 1999).

Complications from hysterectomy are largely minor. However, there are potential long-term complications, namely premature ovarian failure (Siddle et al 1987), sexual dysfunction (Poad and Arnold 1994), cardiovascular disease, bowel function (Parys 1989) and bladder dysfunction (Parys et al 1989; Thacker & O'Keeffe 2000). El-Toukhy et al showed in a prospective study that there was an improvement in all types of incontinence and dyspareunia after hysterectomy by all routes (abdominal total and sub-total, vaginal and laparoscopic (El-Toukhy et al 2003).

Dicker et al published the CREST report in 1982 reporting complications after hysterectomy. Garry and Phillips (1995) and Deprest et al (1995) have also reported. These results are summarised in table 3.2.

Most hysterectomies continue to be performed abdominally in the UK, Australia and USA (Prentice 1999). Complication rates are inevitable with any

surgical procedure. As always, an experienced operator will obtain the lowest complication rates. The abdominal approach is easier than the others but has a higher overall complication rate, although this may reflect the fact that surgeons will choose the abdominal approach for the more difficult hysterectomies. The numbers involved in LH are probably too small to compare but certainly morbidity tends to be lower, but the sequelae of the specific LH complications can be far worse.

Complication rate (%)

Hysterectomy route	Abdominal	Vaginal	Laparoscopic
Unexplained fever	10 – 20	5 – 8	1.4
Operative site infection	13.8 – 42	7.8 – 20	4.3
Urinary tract infection	1.1 – 5	1.7 – 5	<1
Intraoperative haemorrhage	0.2 – 3.7	0.5 – 3.5	N / A
Post-operative bleed	0.2 – 2.3	0.4 – 5.7	0.8
Transfusion	2.2 – 7.5	0.7 – 13	1.2
Bladder trauma	1 – 2	0.5 – 1.5	1.1
Bowel trauma	0.1 – 0.5	0.1 – 0.8	0.5
Ureteric damage	0.1 – 0.5	0.05 – 0.1	0.3
Urinary retention	4.8	8	0.3
Paralytic ileus	2.2	0.2	0.2
Wound dehiscence	0.3 – 0.7	N/A	0.3 (port site hernia)
Vessel injury	N/Q	N/Q	0.4
Atelectasis	6	1	N/Q
Pulmonary embolus	0	0.2	0.2
Myocardial infarction, or cardio-pulmonary arrest	0	0.1	N/Q
Disseminated intravascular coagulation	0	0.1	N/Q
Death	0.2	0.1	0.03

N/Q = not quoted

N/A = not applicable

Table 3.2: Complication rates from hysterectomy (figures from references quoted in text).

An adverse psychological reaction has been long associated with hysterectomy (Polivy 1974). At the turn of the twentieth century a hysterectomy or bilateral oophorectomy were recommended for the treatment of personality disorder and psychiatric illness. Of course this treatment often led to worse psychological problems in the most vulnerable of women (Anath 1978). Depression after hysterectomy is more likely to be due to the considerable hormonal changes than psychological changes. However a considerable amount of the psychological morbidity associated with hysterectomy may be due to pre-existing disease.

In women where the uterus is a symbol of femininity because of menstrual and reproductive capacity, hysterectomy will lead to a feeling of loss of femininity (Hollender 1960; Barglow et al 1965). However, a prospective study failed to show any influence of femininity on post-hysterectomy depression (Gath et al 1987). Meikle et al (1979) studied three groups of women having cholecystectomy, tubal ligation and hysterectomy. They found no differences in mood disturbance between the groups. Martin showed no difference in psychiatric problems before or after hysterectomy (Martin et al 1980). In an Oxford study women were reviewed before and after hysterectomy and a significant reduction was noted at the six-month follow-up in psychiatric problems which more than halved (Gath et al 1987). Another study showed a reduction in women undergoing hysterectomy or endometrial ablation because of dysfunctional uterine bleeding (Alexander et al 1996).

The uterus has been thought of as important to a woman for sexual fulfilment (Poat et al 1994). However, prospective data from studies shows that there

was either no change or improvement in sexual activity. The reasoning behind this being that there is now a freedom from the worry of pregnancy, as well as treatment of gynaecological symptoms such as dysmenorrhoea, which led to the loss of libido in the first instance (Poat & Arnold 1994). Maas et al showed that there were a large majority of women who after hysterectomy had an improved sexual fulfilment (Maas et al 2003), however there were more in the radical hysterectomy group who had a worsening of their fulfilment (Maas et al 2004).

The endometrium has a remarkable capacity to regenerate, and any surgical procedure to treat excessive uterine bleeding, that excludes a hysterectomy must ensure that there is adequate destruction of the basal glands of the endometrium. In the past many methods have been tried in order to cause scarring and to induce an iatrogenic Asherman's syndrome, whether with cryotherapy, steam, intracavitary radioactive substances or other noxious chemicals such as formaldehyde, silicone rubber, quinacrine or oxalic acid (Goldrath et al 1981). None were particularly successful. In the 5 years following referral to a gynaecologist for management of menorrhagia, 81% of women had been admitted to hospital. Forty four percent had had a hysterectomy, 48% had a dilatation and curettage, with 12% having received only drug therapy, and 5% having had no active treatment (Coulter et al 1991).

3.3.2 Uterine curettage.

Uterine curettage is associated with a reduction in MBL in the first few months after the procedure of up to 60% (Mikuta 1970), however the effects are short-lived (Haynes et al 1977). It is a relatively simple procedure to carry

out, but complications include uterine perforation and intrauterine adhesions or synechiae.

3.3.3 Hysteroscopic surgery - endometrial ablative techniques.

There are many techniques available for endometrial destruction. Some are well-established and well researched. Others are still at the research or evaluation stage (Table 3.3). The newer techniques will be discussed in chapter 13 in the discussion of the PDT results.

<u>Hysteroscopic techniques</u>
Transcervical resection (TCRE)
Rollerball ablation
Endometrial laser ablation (ELA)
Hydrothermal ablation (HTA)
Versapoint
<u>Non-hysteroscopic techniques</u>
Radiofrequency ablation (REA)
Microwave ablation (MEA)
Thermal balloon ablation
Electrode balloon ablation
Cryoablation
Photodynamic ablation
Interstitial hyperthermia

Table 3.3 Techniques of endometrial ablation

3.6 Summary.

Prostaglandin inhibitors and antifibrinolytic agents reduce MBL by 25-50%, and are successfully used as a first line treatment in menorrhagia. The LNG-IUS produces a marked reduction in MBL and offers a more acceptable alternative to both oral treatment and surgery. Most of the transcervical MAS techniques for the management of menorrhagia result in an 80% satisfaction

rate, but 1 in 6 of treated women will still undergo a hysterectomy. Complications of these MAS techniques such as uterine perforation and

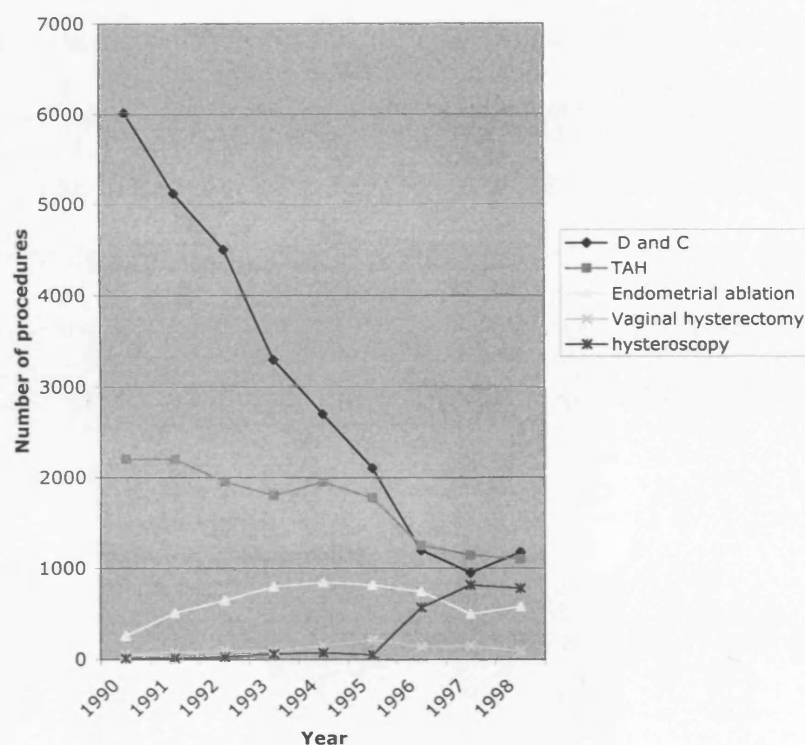


Figure 3.2: Operative procedures for menstrual disorders 1990-99 in women under 50 years (Scotland).

extrauterine organ damage are severe. The overall number of procedures being carried out on women for menstrual disorders is lower now than 10 years ago. The reasons for this are the steep fall in the number of D and C operations, and the increase in the use of hysteroscopy (figure 3.2).

The 'blind' transcervical procedures have, generally, not been thoroughly evaluated. Hysterectomy is the best treatment of menorrhagia, but has a higher complication rate.

CHAPTER 4: LASERS.

4.1 Introduction.

The word laser is an acronym for Light Amplification by the Stimulated Emission of Radiation. Stimulated emission is a physical phenomenon, which occurs in atoms when excited electrons are stimulated by an external energy source to move from a high energy level to a lower level, with the emission of one photon, identical to the one that stimulated the emission. Einstein (figure 4.1) first described this concept in 1917, as part of quantum theory (Patel 1964: World Book 2001). Laser development, however, was dependent upon the development of optics and quantum physics, and did not occur for several more decades.

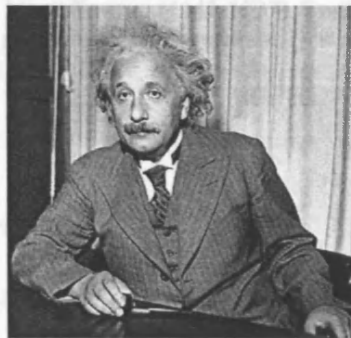


Figure 4.1: Albert Einstein, (1879-1955).

4.2 Theory of light.

In the 17th century Newton (figure 4.2) proposed the 'corpuscular theory' of light considering it to be composed of a stream of particles or corpuscles.

At the same time Hooke and Huyghens considered light to be wave-like and propagated through an all pervading elastic medium called 'æther'. Experimental evidence supported both theories. However in 1802, Young used the wave theory of light to explain the phenomena of diffraction,



Figure 4.2: Isaac Newton (1642-1727), English scientist, astronomer, and mathematician.

polarisation and interference and so the wave theory became generally accepted. In 1867, Maxwell discovered that the speed of propagation of a disturbance in an electromagnetic field was exactly equal to the measured speed of light (measured by and found to be 300 000 km per second). He concluded that light was an electromagnetic wave whose propagation through the 'æther' was determined solely by the electric and magnetic properties of the Æther (World Book 2001).

In 1905, Einstein published his Special Theory of Relativity and showed that electromagnetic waves were self-propagating and had no need of an 'æther'.

Another phenomenon, which could not be explained by the wave theory of light, was the 'photoelectric effect'. Einstein explained this by treating light as being made up of discrete indivisible packets of energy, later to be called photons. The energy of each photon was inversely proportional to the wavelength of the light; therefore ultraviolet light with its short wavelength had high-energy photons. The concept of the photon, in which light is both a particle and a wave, formed the basis of an entirely new branch of physics called the Quantum Theory of Matter.

Niels Bohr (1885-1962), a noted Danish physicist developed a theory about the structure of the atom. Bohr's theory, published in 1913, was based on an earlier one proposed by Ernest Rutherford, a New Zealand-born physicist. Bohr used the Quantum Theory to remodel the atom, suggesting that atoms consisted of positively charged nuclei surrounded by negatively charged electrons in discrete orbits, usually in a low energy state known as the ground state (World Book 2001). The emission and absorption of light results from changes in the energy of the atom, which the quantum theory showed to be restricted to only certain allowable values, each of which could be represented by a particular configuration of the extra-nuclear electrons. A change from one energy state to another was instantaneous and accompanied by the absorption or emission of a packet of energy (or photon) equal to the difference in energy between the two states, the transition energy. If a photon of an appropriate wavelength collides with an atom it is absorbed, the energy is transferred to one of the orbiting electrons, which will move into a higher more energetic orbit. The excited electron, and therefore the atom, is now in an unstable, excited state and its tendency is to return back to the ground state by re-

emitting the excess energy as a photon. If enough energy is added to the atom the electron becomes detached and the atom is ionised. Quantum Theory will only allow photon absorption to happen if the absorbed photon has exactly the same energy as that required to move the electron between permitted energy levels. Similarly, the photon emitted following a return to the ground state possesses the same energy.

Einstein called the first process, absorption and the second, spontaneous emission. He also suggested a third process: if an already excited atom is struck by a photon of the correct wavelength this will stimulate the emission of a second photon of identical wavelength, in phase, and with a parallel path to the incident photon. This process is called stimulated emission and results in an amplification of the original photon by a factor of two.

4.3 Early laser development.

The practical significance of Einstein's theory was not realised until after the Second World War, when it was found that conventional electronic devices were unable to amplify the very high frequency microwaves used for radar. Basov, Prokharov and Townes realised that the intensity of the original electromagnetic wave could be amplified by the phenomenon of stimulated emission. For this to occur there must be a situation whereby more than 50% of the atoms have electrons occupying a higher energy level called a 'population inversion'. These excited atoms are 'prepared' to release their energy as photons. Without such a high number of atoms in the excited state, photons would be absorbed by the lower level atoms rather than stimulate emission of further photons from the upper level (Gordon et al 1955). In 1953 Charles Townes produced such a population inversion and constructed the

first 'MASER': an acronym for Microwave Amplification by Stimulated Emission of Radiation. Shortly after this in 1958 Townes and Arthur Schawlow described the conditions necessary for Light Amplification by Stimulated Emission of Radiation (LASER). For his work, Townes shared the 1964 Nobel Prize in physics with two Soviet scientists who also developed and improved masers (World Book 2001).

A laser consists of three basic elements, a power supply, the excitable medium and an optical resonator. The atoms or molecules in the medium are raised to a higher energy by the power supply. Many substances are suitable for use as the lasing medium. They may be solids, liquids, gases or metal vapours (Thearruth and McKenzie 1986).

4.4 The ruby laser.

Theodore H. Maiman a physicist at the Hughes Research Laboratories in the United States constructed the first laser in 1960. His laser used a ruby rod as its active medium. He demonstrated lasing action using a small rod of synthetic ruby crystal as the laser medium on May 16, 1960 (Maiman 1960). Ruby is predominantly aluminium oxide (Al_2O_3) with a small percentage of chromium oxide (Cr_2O_3) held within the crystal lattice. Its pale pink colour is caused by absorption of light in the green and violet parts of the spectrum by the chromium (Cr^{3+}) ions. The rod shaped ruby crystal was silvered at both ends so that light travelling parallel to the longitudinal axis would be reflected back and forth through the ruby rod. To allow some light to escape one face was only partially silvered. The energy needed to excite Cr^{3+} ions and produce the population inversion necessary for laser action was produced by a xenon flash discharge lamp.

Cr^{3+} ions are excited to high energy levels by the absorption of green and violet light emitted by the flash lamp. The absorption bands are very short lived and decay almost immediately to a metastable state via non-radiative transitions. The transition energy is lost as heat in the crystal lattice. The metastable state is relatively long lived and returns to the ground state via radiative transitions, which emit red light (wavelength 694nm). Because the absorption bands decay very rapidly to a longer lived metastable state, a population inversion between metastable and ground state is achieved and laser action is therefore possible.

Laser action is initiated by a photon emitted by the spontaneous decay of an excited Cr^{3+} ion and this travels parallel to the longitudinal axis of the rod. Along its path, the photon stimulates the production of another photon, which propagates in the same direction. The two resultant photons stimulate the emission of more identical photons by further interactions with other excited ions. The chain reaction, of which these are the first steps, continues as photons travel back and forth through the ruby rod and lasts for as long as the population inversion remains. The result is an intense pulse of highly collimated red light emerging from the partially reflective face of the rod.

Following the invention of the ruby laser, a number of other lasers in the visible and infrared spectrum have been designed. The neodymium yttrium aluminium garnet laser (Nd:YAG laser) was first demonstrated in 1961 (Johnson 1961), the argon ion laser in 1962 (Bennet et al 1962) and the carbon dioxide laser in 1964 (Patel et al 1964). The first ultraviolet laser, using excited molecular xenon was demonstrated in the USSR in 1970 (Basov et al 1970).

In 1961 the American physicist Ali Javan constructed the first gas laser. In 1962, three separate teams of U.S. scientists operated the first semiconductor lasers. In 1966, the American physicist Peter Sorokin built the first dye laser. The carbon dioxide (CO₂) laser was reported in 1964 (Patel 1964) and is still widely used in clinical practise as is the neodymium-YAG (solid state laser) (Geusic et al 1964) and diode laser (Ripley 1996).

4.5 Properties of laser light.

In summary there are four basic characteristics of light produced by a laser, in which it differs from ordinary light. Namely that it is monochromatic, coherent, collimated and highly directional. Lasers are also capable of producing high powers and power densities.

4.5.1 Monochromaticity.

Laser light is monochromatic consisting of one wavelength. It is one colour because all the photons come from one transition between two energy levels.

4.5.2 Coherence.

Ordinary light from a lamp is 'incoherent' and consists of a mixture of wavelengths radiating in all directions. Laser light is 'coherent' and consists of one wavelength with all its waves travelling in the same direction and 'in phase' with each other. Waves are in phase such that all troughs and all the peaks are exactly opposite one another.

4.5.3 Collimation.

A collimated beam is one that is parallel and does not diverge. This is unlike light from a light bulb, which spreads out as it travels further away from its

source. An important property of a highly collimated laser beam is that although the power output of the laser may be low, the irradiance (power per unit area) of the beam is very high and can remain so up to very great distances. The low divergence of the laser beam allows it to be focused to a very small spot size and thus increase irradiances even further. The low beam divergence of laser light is caused by its high spatial coherence.

The monochromatic nature of laser light is important for photodynamic therapy (PDT) as a specific wavelength of light is required for sensitiser activation. All of the light produced shares the same optical properties. This improves efficiency, and enables the light to be channelled down an optical fibre. The highly directional nature also allows the beam to be focused into a very small spot, resulting in a high concentration of energy.

4.6 Lasers suitable for clinical use.

A substance has the potential to become a lasing medium if it can have more atoms or molecules in a high-energy state than a lower energy state - population inversion. In most lasers, a gas, liquid or crystal is energised (pumped) by a suitable energy source (light, electric discharge, radiofrequency emitter). The input of pumping energy raises electrons to higher energy levels in more atoms, more quickly than spontaneous decay can return them to their original level. Once there are more excited atoms (i.e. atoms having an electron in a higher energy level), stimulated emission becomes possible.

All lasers consist of a chamber that has mirrors at each end so that light can be reflected back and forth within the chamber. One of these mirrors will have either a hole at the centre or be only partially reflective so that the laser beam

can leave the chamber. The light can then be focused by a lens and passed into a suitable delivery system. The laser medium determines the range of wavelengths and the mirrors determine the specific wavelength of the laser light.

4.6.1 Argon laser.

The laser medium of the argon ion laser is argon gas held in a small diameter air or water-cooled tube made of glass, graphite or beryllium oxide. An electric current ionises the argon gas and excites the ions to high-energy states. The laser produces blue-green light and this output is made up of a number of wavelengths lying between 437-529nm (80% of the power is equally divided between 488 and 514.5nm). The argon ion laser is very inefficient; it needs a three-phase electrical power source and a supply of cooling water. The blue-green light from the argon ion laser passes through skin with minimal absorption and is preferentially absorbed by certain tissue pigments such as melanin and haemoglobin. This laser is used much less now because of the use of semi-conductor lasers which are more reliable and efficient.

4.6.2 Carbon dioxide laser.

The carbon dioxide (CO₂) laser is one of the most commonly used surgical lasers. It emits energy in the non-visible portion of the light spectrum (far infrared) with a wavelength of 10,600nm. This light is strongly absorbed by water and all conventional optical fibres. The laser medium of the CO₂ laser is a mixture of carbon dioxide, nitrogen and helium gas, held in an air or water-cooled discharge tube. The CO₂ laser has a high efficiency of 15% and can be

powered by a single-phase electrical source and does not need an external supply of cooling water. As the laser beam is invisible, it is used with a second laser, a visible coaxial (red) helium-neon aiming beam. It is used in gynaecology in the treatment of cervical intraepithelial neoplasia and at laparoscopy.

The output of the CO₂ laser is strongly absorbed by all conventional optical fibres and the only flexible fibres currently available to transmit the CO₂ beam are toxic, expensive and relatively inefficient. This limits the use of the CO₂ laser to rigid instruments, which are manoeuvred, with a series of articulated arms.

4.6.3 Neodymium Yttrium Aluminium Garnet laser.

The laser medium of the neodymium yttrium aluminium garnet (Nd-YAG) laser is a synthetic crystal of yttrium aluminium garnet which has been 'doped' with a small concentration of neodymium atoms. Light from a krypton lamp pumps the neodymium atoms into a broad band of high-energy states which rapidly decay via non-radiative transitions to long lived metastable states. Lasing occurs at the near infra-red 1,064nm wavelength.

One way of achieving population inversion is to use materials in which the laser transition is not between a pumped and the ground state but between two other energy levels. This is the case with the Nd-YAG laser, which is a four level laser system. The pumping energy raises some of the Neodymium into a higher (E3) energy level. This decays rapidly to the E2 level and the laser action occurs between the E2 and E1 levels with the latter decaying rapidly to the ground (E0) state. Because the laser transition is slower than the others,

population inversion between the laser levels (E2 and E1) can occur with the vast majority of particles still in the ground state (Geusic et al 1964).

The Nd-YAG laser is usually run as a continuous wave system. However using a Q-switch can produce very short, high-powered pulses. This is a shutter, which is placed inside the laser cavity preventing the normal movement of light, and thus inhibiting stimulated emission. The laser medium in a switched laser can be pumped to an energy level far greater than one which would normally induce a population inversion and thus stimulated emission. Therefore when the shutter is opened, the energy stored within the laser medium is released as a single optical pulse with duration of about 10 nanoseconds and a peak power of a gigaWatt (10^9 Watt).

The efficiency of the Nd-YAG laser compared to other lasers is relatively low. Most Nd-YAG lasers require a three-phase electrical power source and a supply of cooling water, although newer designs can run on single-phase electricity without external cooling water. To enable visualisation of the path of the invisible infrared beam, a coaxial visible red beam of a low power helium-neon laser is used. Both the visible red and near infrared beams can be transmitted by small diameter optical fibres.

4.6.4 Potassium-titanyl-phosphate laser.

The potassium-titanyl-phosphate (KTP) laser produces green light of wavelength 532nm, which can be transmitted along flexible fibres. This laser is simply an Nd-YAG laser whose beam is passed through a KTP crystal, which doubles the frequency and so halves the wavelength. It is used laparoscopically in the treatment of polycystic ovarian disease and for ovarian

endometriomas. Like the argon laser it produces more tissue coagulation than the CO₂ laser but less than the Nd-YAG.

4.6.5 Dye lasers.

Dye lasers have the advantage of producing light with a large range of tuneable wavelengths. The laser medium is a dye, such as coumarin or rhodamine, in a solvent like ethanol. A typical dye is able to lase at any wavelength within a continuous range of about 70 nm and different dyes produce different ranges. The efficiency of a dye laser is relatively high at 25% but it needs to be driven by another laser (e.g. argon or copper vapour laser). These lasers are used in photodynamic therapy.

4.6.6 Metal vapour lasers.

A number of metals can be made to lase when in their vapour phase. They include copper, which lases at 511 nm (green) and 578 nm (yellow), and gold, which lases at 628 nm (red). Metal vapour lasers can only operate in a pulsed mode with a high repetition rate. These lasers are used in photodynamic therapy.

4.6.7 Excimer lasers.

Excimer is a contraction of 'excited dimer' and these lasers provide a source of ultraviolet light. The laser medium of an excimer laser is a volume of gas in which the atoms will only form dimers when in an excited state. These excited molecules will immediately dissociate and release high-energy ultraviolet light on returning to the ground state. The first excimer laser used excited dimers of xenon (Xe₂). More modern lasers use a rare gas atom (argon, krypton, and xenon) combined in the excited state with a halogen atom (fluorine, chlorine,

bromine). Examples of rare gas-halide excimer lasers are argon fluoride with a wavelength of 193nm and krypton chloride with a wavelength of 222nm. Excimer lasers can be pumped either by an electron beam or by an electrical discharge. The output is pulsed and the length of each pulse is about 10-20 nanoseconds. The beam can be transmitted along a fiberoptic system only at wavelengths above about 300nm. These lasers are still in the experimental stages, in certain cases such as in angioplasty. In ophthalmology they are becoming established for reshaping the cornea for the correction of short-sight.

4.6.8 Diode Laser.

The diode laser is formed from a tiny chip of gallium arsenide semiconductor material. This converts electricity to laser light with a high efficiency of 30% thus reducing power demands and heat generation. The tissue effects of a 805nm diode laser are similar to those produced by a Nd-YAG laser at 1064 nm. Its main advantages, however over the Nd-YAG laser are that it is easily portable, maintenance and service free and needs no special electrical or plumbing requirements. It has been claimed that the diode laser can be used in all techniques, which previously required a Nd-YAG laser (Wyman et al 1992).

4.7 Laser Light and Living Tissue.

4.7.1 Light Tissue Interactions.

When a photon of light hits a tissue surface, four interactions can occur and are summarised as follows:

1. Reflection at the tissue surface - due to the difference in the optical properties (refractive index) of air and tissue.
2. Transmission through the tissue (no interaction).
3. Scattering within the tissue- by small particles such as mitochondria in cells and at cell interfaces.
4. Absorption by the tissue.

This latter effect can lead to the production of heat. By the appropriate adjustment of laser wavelength, certain chromophores (light absorbing molecules within the tissues) can be targeted for clinical use e.g. intracellular water is highly absorbing for CO₂ radiation and therefore can be used for cutting or vaporisation and is the mechanism seen with thermal surgical lasers (Carruth 1984). Alternatively, the wavelength can be used to target photosensitisers and cause a photosensitised change to the cell (photodynamic therapy). If it is reflected from or transmitted through the tissue no effect will occur. If the light is scattered, it will be absorbed over a larger volume so that its effects will be more diffuse (Parrish et al 1985). Only absorbed light can produce a biological effect.

However unlike ordinary light, laser light has several special qualities. It is highly collimated so the laser beam can be focused to a very small spot size and very high irradiances can be achieved and large amounts of energy can be

deposited very precisely in the tissue. It is also monochromatic light, which can penetrate varying distances into tissue according to its wavelength. Ultraviolet light (193-400 nm) is very strongly absorbed. Visible light (400 - 700 nm) becomes more weakly absorbed as its colour changes from blue to red and in the near infrared (around 1000 nm) tissue becomes relatively transparent. Further into the infrared spectrum, absorption again increases above 1300 nm. The CO₂ laser has a wavelength of 10,600 nm and is very strongly absorbed in water.

4.7.2 Light penetration into tissue.

The depth of light penetration in tissue is a very important feature in photodynamic therapy, as the action of light on the photosensitiser is crucial to the destruction of tumours. The depth of light penetration through different tissues is a function of the optical properties of that tissue, specifically absorption and scattering coefficients and the scattering angular distribution. These factors are related to the tissue chromophores in addition to the wavelength of the laser light itself.

The opacity of a tissue is defined as the attenuation coefficient whilst its inverse is the penetration depth of light, which is the depth when the incident light intensity drops to approximately 37% of the initial value ($1/e$). Penetration depths into tissue typically range from 1-2mm to nearly 5mm at a wavelength of 630nm used for sensitiser activation with Photofrin and PPIX (Dougherty & Marcus 1992), while the penetration depth is almost doubled at 700-850nm (Wilson et al 1986). This improved penetration depth at longer wavelengths has prompted the development of sensitisers that absorb in this region. Examples of such sensitisers include, benzoporphyrin derivative which

absorbs at 690nm, the chlorins (652-660nm) and the naphthalocyanines which absorb strongly at 760nm (Dougherty & Marcus 1992). However, absorption of light by the photosensitiser itself can limit tissue penetration and is particularly pronounced with high concentrations of sensitisers, which act as very strongly absorbing chromophores at the treatment wavelength, (Wilson et al 1986).

4.7.3 Thermal effects of laserlight.

Lasers deliver energy to tissue that is absorbed as heat, so their most common clinical effects are thermal. Regardless of the laser system, laser light that has been absorbed by tissue may cause hyperthermia, coagulation and vaporisation, effects which are temperature dependent (Welch 1984).

4.7.3.1 Hyperthermia.

This process involves gentle heating of the tissues to produce cell death without major alterations in tissue architecture (Matthewson et al 1987). Tissue temperatures usually remain within the subcoagulation range, which is less than 60°C.

4.7.3.2 Coagulation.

Temperatures below boiling point can be used to treat large volumes of tissue, the Nd-YAG laser is particularly effective because it has a greater ability to penetrate tissue than other lasers such as the CO₂.

4.7.3.3 Vaporisation.

If the tissue is allowed to heat to greater than 100°C, boiling takes place within the intracellular water. The rapid rise in intracellular temperature and pressure causes cellular explosion creating steam and cellular debris. This steam and

debris is seen as the laser plume. The plume remains in the path of the laser beam and the particle fragments flash white-hot as they are carbonised.

Three types of thermal laser are in widespread clinical use: the CO₂ laser, the Nd-YAG laser and the KTP laser. The CO₂ laser light is strongly absorbed in water, whereas the other two are absorbed more in pigmented cells (Fenech et al 1985). Any laser of sufficient energy to be absorbed at the tissue surface results in the superficial cells being destroyed and further application of the beam will bore a hole in the tissue (Garrison and Srinivasan 1985). However beneath the area of vaporisation there is a zone of coagulation which varies in depth depending on the laser used. The CO₂ and Nd-YAG lasers cause coagulation to a depth of 0.1mm and 5mm respectively (Litwin and Earle 1965).

Initially, heating of soft tissue causes thermal contraction of the treated area and as tissue shrinks, small vessels are sealed, which can stop haemorrhage. Thrombosis in occluded vessels only occurs as a secondary effect. The Nd-YAG laser can heat large volumes of tissue and can therefore seal vessels with a diameter up to 1mm in suitable supporting tissue. The volume heated by the CO₂ laser outside the area of vaporised tissue is so small that it has little effect on major haemorrhage, although it can seal capillary oozing particularly when a large defocused spot is used. The CO₂ laser has a highly localised effect on tissue and therefore acts as a laser knife with the cells immediately under the beam becoming vaporised with minimal damage to adjacent areas.

4.7.4 Non-thermal effects of laser.

Light can produce non-thermal effects in tissue, which include photoablation and the activation of photochemical reactions in photosensitive dyes (the basis of photodynamic therapy). Photoablation occurs when using light with a short wavelength (ultraviolet) because the photon energy may be great enough to directly disrupt molecular bonds within tissue. The excimer laser is said to exert its effect by means of photoablation.

4.7.5 Photodynamic therapy.

Photodynamic therapy (PDT) involves the pre-treatment of target tissues with photosensitising agents which enable light within the visible or infrared region up to about 1000nm to produce a severe cytotoxic effect. It has been observed that certain tissues, particularly tumours, selectively retain the photosensitiser while adjacent tissue areas contain very little (Gomer & Dougherty 1979), although this selectivity has often been over emphasised (Bown 1990). Light at a specific wavelength corresponding to an absorption peak of the photosensitiser is then used to activate this drug to produce local necrosis in the required area and leave adjacent tissue undamaged.

4.8 Laser and light delivery systems.

Delivery of light allowing optimal irradiation of the whole target tissue is essential to the success of photodynamic therapy. The distribution of light must not only match the geometric but also the optical characteristics of the target tissue, in order to optimise tumour kill and minimise damage to the normal surrounding tissue (Wilson & Patterson, 1986). The light source used for PDT must be capable of providing the appropriate wavelength of light

matched to the sensitiser to be used, of an adequate intensity to allow treatment to be carried out in a reasonable length of time and be coupled to an appropriate light delivery system (which can be selected for different geometries).

4.8.1 Light sources.

Various types of light sources are available for photodynamic therapy as lasers are not essential. Historically sunlight has been used (von Tappeiner and Jesionek 1903), whilst wavelength filtered lamps have been used in the past with a recent revival of interest due to low cost production, running cost and size (Dougherty et al 1975; Morton et al 1995)

Before the development of diode lasers, the most commonly used lasers for PDT were tuneable dye lasers, which are wavelength conversion devices. The dye laser is ‘pumped’ by another laser and converts the energy from the pump laser by the use of fluorescent dyes such as rhodamine. This results in a tuneable output over a range of 30-50 nm (620-650 nm), and such devices have been used extensively in PDT related research. The advantage of dye lasers is their tunability over parts of the visible spectrum. Copper vapour and argon ion lasers are frequently used as the ‘pump laser’ due to strong absorption by rhodamine dyes at the green wavelength. The dye can be changed to provide different range of wavelength.

The copper and gold vapour lasers are bulky and too expensive for most hospitals to purchase specifically for PDT. However, many surgical units already have a potassium titanyl phosphate (KTP) Nd:YAG laser that can also be used to pump a dye module. The ultimate ‘black box’ laser for clinical use

must be the diode lasers which make use of semiconductor technology to emit light with high power output and are currently available at, for example, 630, 652 and 675nm for use with ALA, mTHPC and phthalocyanines. These compact size lasers are not only portable but significantly cheaper and more user friendly than the other laser systems making them particularly attractive for PDT use in non specialised units who do not have easy access to technical support. The disadvantage of the diode lasers is their lack of tunability, which means that extra systems would need to be acquired if using more than one sensitiser at different wavelengths.

There has also been renewed interest in non-laser sources particularly for dermatological applications. A lamp incorporating a 300W xenon short arc plasma discharge with a bandwidth filter of 30nm has been developed (Whitehurst et al 1993; Whitehurst et al 1995). Such light devices may further complicate light dosimetry, as a percentage of the incident light may not be of an appropriate wavelength to activate the sensitisers especially with sensitisers that have very narrow absorption bands. Consequently more energy may be required than with laser light to achieve the same PDT effect, the presence of infrared light will further complicate matters.

4.9 Lasers used in gynaecology.

Lasers have been used quite extensively in the past for various conditions. They are now virtually never used, except at laparoscopy.

The CO₂ laser was first used in 1973 for the treatment of cervical lesions by vaporisation (Kaplan et al 1973). Unlike diathermy or cryosurgery there was little post-operative pain. Treatment of cervical intraepithelial neoplasia (CIN)

has cure rates of more than 90% (Berget 1987). Laser treatment has the advantage of destroying or removing less tissue, with more than 95% success rates (Anderson 1982). The main disadvantage of laser is the lack of a histological specimen.

The CO₂ laser has also been used for vaginal intraepithelial neoplasia (VAIN) (Graham & Meigs 1952). It was also used with great effect for vulval intraepithelial neoplasia (VIN) (Baggish and Dorsey 1981). The depth of VIN removal with the laser is 1mm in non-hairy skin and 2mm in hairy skin (Shatz et al 1989). Success rates of 90% are quoted.

4.10 Laser safety.

All lasers on the University College Hospitals sites are used under the supervision of a physicist and a Laser Protection Adviser (who may be the same person). The responsibilities of these persons are several. These include drawing up local rules on laser use, certification of laser operators, planning new areas for laser use or modifying existing areas, recording use of laser and serving as a contact for any questions.

Lasers are dangerous for two main reasons, the output from the laser and the power source used to create the output.

4.10.1 Optical safety.

The Bureau of Radiological Health (1975) classifies lasers based on the potential hazards. A laser using visible light will be a hazard because if shone into the eye, the beam will be focused on the retina by the lens and coagulate it if of sufficient energy. Ultraviolet lasers are heavily absorbed by the cornea

causing damage. Class I lasers are virtually harmless whereas Class IV are damaging at all exposures.

4.10.2 Skin safety.

Exposure of the skin to high-power lasers will cause burning. In a conscious surgeon this results in rapid withdrawal of the affected part. However, if the optical fibre is damaged the patient may be at risk.

4.10.3 Electrical safety.

High voltages are required to energise the laser. This is often facilitated with capacitors within the device. The capacitors may store the voltage for days; they remain dangerous unless the electricity is discharged.

The BRH classifies all therapeutic lasers as Class IV and dangerous. Their use is restricted to a designated Laser Controlled area. To protect the eye, different goggles are worn to protect against different wavelengths.

All safety rules were rigorously applied and observed during experimentation in this thesis.

CHAPTER 5: PHOTODYNAMIC THERAPY.

5.1 Introduction.

Light is electromagnetic radiation in the 250-900 nm wavelength. Ultraviolet light is 200-400 nm, visible light is in the region 400-750 nm, and infrared beyond 750 nm. Light may originate from many different sources, from the sun to lasers of specific wavelength.

Phototherapy is the use of light in the treatment of disease. Phototherapy involves a light-activated chemical process. An example is the treatment of neonatal hyperbilirubinaemia with ultraviolet (UV) and visible light, where no drug is used.

Photochemotherapy is a subdivision of phototherapy and involves the absorption of light energy by an exogenous molecule, which enters a higher energy state. This activated molecule then imparts its energy to tissues and causes the desired therapeutic action (Epstein 1990). An example of this is the use of a psoralen in combination with ultraviolet light (UV-A) in the treatment of psoriasis.

Photodynamic therapy (PDT) is a type of photochemotherapy. PDT is a technique based on the interaction of a drug (photosensitiser) with incident light of an appropriate wavelength, in the presence of tissue oxygen (Foote 1984). This results in a photochemical reaction, resulting in the formation of singlet oxygen or other high energy oxygen radicals, which react with tissue components to cause cellular damage (Bonnet 1999). This damage can be by either direct cell killing or indirect effects (for example by direct effects on the

blood vessels). The photosensitiser is administered either systemically or topically. After a delay (the drug-light interval) the target tissue is irradiated with light, and a maximal destructive effect is obtained in the target tissue with less effect on adjacent tissues.

5.2 History of photodynamic therapy.

Light has been used as a therapeutic tool for centuries, dating back to the ancient civilisations in Egypt, India and China. Light from the sun was used to treat such conditions as vitiligo, rickets, psoriasis, cancers and even psychiatric conditions (Spikes 1985; Epstein 1990).

In the sacred Indian book Atharva Veda dating from 1400 BC, the 'cure' for leprosy and vitiligo was the ingestion of certain black seeds (now thought to be from the plant *Bavachee*, a species known to contain psoralen), together with *Bringaraga* (*Ecclipta prostrata*), *Indravaruni* (*Colocynthis*) and tumeric (*Curcuma longa*) then to expose the affected area to the sun (Fitzpatrick et al 1958, Daniell and Hill 1991). Herodotus first documented the healing properties of light and developed heliotherapy (Daniell and Hill 1991). Hippocrates in the fourth century BC advocated the use of the solarium for building up wasted muscles (Bonnett 1999).

Modern heliotherapy reappeared in Switzerland at the start of the twentieth century. It was used by Bernhard and Rollier for the treatment of the skin manifestations of tuberculosis (Rollier 1927).

Phototherapy is used in the treatment of jaundice in the newborn, and was introduced in the late 1950s (Cremer et al 1958). Bilirubin is a neural toxin and the only treatment available prior to this discovery was for an exchange

transfusion. There are actually two mechanisms involved; photosolubilisation, whereby bilirubin is converted to soluble photoisomers, and photofragmentation in which the bilirubin is photo-oxidised to soluble by-products which are then excreted (McDonagh and Lightener 1985).

Niels Finsen, a native of the Faroe Islands, trained and worked in Copenhagen. He advocated the use of light devoid of ultraviolet radiation (ie red light) to reduce the scarring from smallpox. He also showed that light from a carbon arc light could be used to treat lupus vulgaris - the skin manifestation of tuberculosis. This condition was almost endemic in Scandinavian countries and he was acclaimed. The Finsen Medical Light Institute was set up and he was awarded the Nobel Prize in 1903 for his work on phototherapy. Queen Alexandra brought the idea to London from Denmark and as President of the London Hospital set up the Light Department in the early 1900s. This department was still functioning in the late 1920s.

The modern history of Photodynamic therapy can be traced back to the turn of the century. A German medical student, Oscar Rabb, working under von Tappeiner, was able to photosensitise paramecia with acridine dye and kill them with light (Rabb 1900). His experiments were initially to record the behaviour of the paramecia, however, he noticed that on normal days the paramecia were all killed, yet on overcast days they were not. By setting up a series of experiments he showed, in a seminal piece of work, that it required the presence of both light and the acridine to cause death in the paramecia. von Tappeiner recognised that oxygen was also an essential requirement and coined the term 'photodynamische Wirkung' - photodynamic effect (or action). Later, in 1903 von Tappeiner and Jesionek, a dermatologist, published

the first clinical study using PDT. They used topically applied eosin activated by an arc lamp to treat skin cancer successfully (von Tappeiner and Jesionek 1903). However, it was almost 60 years before PDT was used to treat cancer again.

Extensive research on psoralens began in 1941 by Fahmy in Egypt. He isolated several furocoumarins from a powder called 'Atrillal', derived from the fruits of *Ammi magus* a weed growing along the Nile used by herb doctors. This was used to treat vitiligo. The results of a clinical trial were encouraging (El Mofty 1952), and soon after two of the compounds were identified as 8-methoxypsoralen and 8-isoamylene-oxypsoralen were marketed by an Egyptian firm. Confirmation of these results followed in France (Sidi and Bourgeois-Gavardin 1952), and the USA (Lerner et al 1952).

Lipson used haematoporphyrin derivative (HpD) as a photosensitiser for PDT in a case of recurrent breast cancer on the chest wall. HpD is a complex mixture of blood derived porphyrins. The chest lesion showed some response but was not cured (Lipson 1966). Apart from another case report of PDT being used, this time in bladder cancer (Kelly et al 1975), it was not until 1976 that the first studies were carried out using PDT by Dougherty and co-workers (Dougherty et al 1978). Parrish et al (1974) showed that taking oral methoxypsoralen and then using UV-A exposure to the affected skin could treat psoriasis; this is now known as PUVA (psoralen-UVA).

5.3 Mechanisms Of Photosensitisation.

On absorbing light of the appropriate wavelength, the sensitizer is converted from a stable electronic structure (S^0 , the electronic ground state) to an excited state known as the singlet state (S^1*).

S^1* is short lived, being consumed by other processes, and may undergo conversion to a longer-lived excited state known as the triplet state (T^1*), which is the photoactive state responsible for the photochemical generation of cytotoxic species.

S^1* can convert back to the ground state, decaying and giving off light – known as fluorescence, or by giving off the energy as heat.

The lifetime of the singlet state is generally less than 1 microsecond (μ s) and the main role of this state in the photosensitisation mechanism is to act as a precursor of the metastable triplet state. The excitation efficiency is defined as the triplet state quantum yield (ϕ_T), which for photosensitisers should ideally approach unity, i.e. one triplet state formation per absorbed photon. Interaction of the metastable triplet state (which in oxygen deplete solutions has a lifetime extending to the millisecond range) with tissue components may proceed via either a type I or II mechanism (or a combination). A type I process can involve hydrogen abstraction from the sensitizer to produce free radical.

The type II mechanism, in contrast, exclusively involves interaction between molecular oxygen and the triplet state to form an electronically excited state of O_2 known as singlet oxygen (1O_2*), which is highly reactive in biological systems. It is widely thought that the type II mechanism underlies the oxygen-dependent photocytotoxicity of sensitizers used for photodynamic therapy.

A number of the biomolecules in cell membranes react rapidly with singlet oxygen. Cell membranes - and mitochondrial membranes in particular - are prime targets for PDT damage and cell death (Gomer et al 1988).

When the sensitiser transfers electronic energy to O_2 it returns to its ground state. Thus the cytotoxic singlet oxygen species is produced without chemical transformation of the photosensitiser. It is then free to absorb another photon and repeat the cycle. A single photosensitiser molecule is capable of generating many singlet oxygen molecules, provided the oxygen supply is adequate.

However, there are two thresholds, which must be exceeded before a PDT effect can be produced. A minimum tissue concentration of photosensitiser is needed, as below this threshold level the photosensitiser may be inactivated by photodegradation before sufficient singlet oxygen is produced to cause any biological damage. Photosensitisers generally possess long-lived triplet states with high triplet-state and singlet oxygen yields; yet these same chemicals are prone to photodegradation caused by 'auto-oxidation' by the singlet oxygen.

Finally, the product of the total light energy absorbed at each point and the tissue concentration of photosensitiser at the time of light exposure is important. If the concentrations of photosensitiser in a specific tissue layer is too low there will be no PDT damage, whatever the light dose.

Photosensitisers tend to have selective uptake by proliferative and therefore neoplastic tissues. This allows targeting of these tissues for successful PDT, for example in tumours and in the regenerating endometrium. In general all photosensitisers have a strong affinity for tissues with a high reticulo-

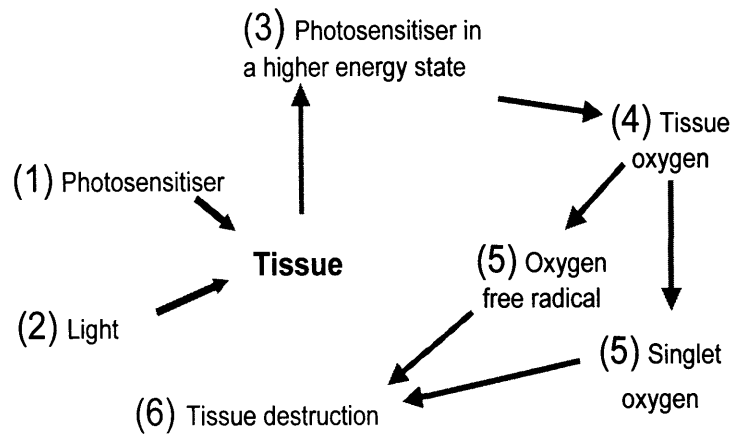


Figure 5.1: Mechanism of photodynamic therapy

endothelial component (Henderson and Dougherty 1992). A simplified mechanism of PDT is outlined in figure 5.1.

Different photosensitisers have different time intervals between administration and peak tissue levels. Indeed, this may also vary between tissues. The time for metabolism or excretion is also highly variable, lasting from hours to some weeks.

5.4 Mechanisms of tissue destruction.

5.4.1 Cellular targets of PDT.

Subcellular sites of tissue damage caused by PDT include the plasma membrane, many organelle membranes and in particular the mitochondria

(Gomer et al 1980). There is evidence of direct DNA damage, but this is not a determinant of cytotoxicity (Fisher et al 1995). Apoptosis is also induced by PDT and is probably induced via the cell membrane. However the significance of apoptosis compared with necrotic cell death in the PDT effect is not clear (Fisher et al 1995).

5.4.2 Vascular destruction versus direct cell death.

van Geel et al (1996a) showed that vascular injury was a major factor in the efficacy of PDT in mouse tumour destruction. Endothelial cells and macrophages are known to be highly sensitive to photosensitisation. Irradiation of these sensitised cells will cause the release of various vasoactive and inflammatory agents, which affect the microvasculature in a target organ (Henderson and Donovan 1989).

5.5 Photodegradation (photobleaching) of the photosensitiser.

Photosensitisers are generally stable, but will tend to degrade in response to light irradiation. Thus, during PDT, the concentration of active photosensitiser decreases (Wyss et al 1995). The efficiency of PDT is, therefore, maximal at the beginning of light exposure. Photobleaching occurs maximally at the surface where the fluence rate is higher. There is therefore a finite amount of singlet oxygen that a given amount of photosensitiser can produce.

5.6 The development of photosensitisers.

In the early part of the twentieth century, Oscar Raab had used acridine dye in his seminal work with paramecia. von Tappeiner had conducted the first clinical trials with eosin. This work was extended to include other sensitisers

with similar results. Policard in 1924 noticed that there was a (presumed) porphyrin fluorescence seen in experimental tumours. This association between porphyrins and cancers was shown by experimental tumour photonecrosis by Auler and Banzer in 1942 in Berlin. In the late 1940s Figge and co-workers showed that haematoporphyrin selectively localised in experimental tumours. It was not until Lipson et al introduced haematoporphyrin derivative (HpD) that PDT started to have an impact on the scientific and medical community (Lipson 1964). Lipson and his colleagues used HpD not for treatment but more for diagnosis (Lipson et al 1964; Kyriazis et al 1973). This led to the development of fluorescence endoscopy, and photodiagnosis.

Diamond et al demonstrated photonecrosis of a tumour in a rat in 1972. They showed that PDT was of possible therapeutic importance. Diamond was the one who reintroduced the term photodynamic therapy into use.

Several centres then started to investigate HpD and other photosensitisers. Dougherty et al made the biggest breakthrough in 1975. They showed a 48% cure rate in transplanted animal tumours treated with HpD-induced PDT. Dougherty followed this up with a study looking at PDT for tumours in 25 patients at several sites including breast, prostate, colon and skin. He stated that 'no [cancer] type has been found to be unresponsive' (Dougherty et al 1978).

The commercialisation of HpD and its derivatives has proceeded in various countries. The most significant development has been with Photofrin (QuadraLogic Technologies Inc, Vancouver, Canada), which was approved for the treatment of bladder cancer in Canada in 1993. This was followed by

approvals in Japan, the USA, France and the Netherlands for a variety of cancers (e.g. lung, oesophagus, stomach, cervix, bladder).

HpD and its derivatives were termed 'first generation' photosensitisers, as newer 'improved' drugs became available. However, only ALA is approved by the FDA in topical use for actinic keratosis. Foscan is approved for use in palliation of advanced oral cancers.

Most of the second-generation photosensitisers belong to the tetrapyrrole class. The advantages of this is that these substances are usually completely inert in the absence of light, they are stable and generally have intense absorption peaks in the red part of the visible spectrum which is where tissue transmission is most efficient and they are efficient generators of singlet oxygen.

5.6.1 Porphyrins.

Several porphyrins have been examined. For example, 5,10,15,20-tetrakis(m-hydroxy-phenyl)porphyrin (m-THPP) was found to be 25-30 times more potent than HpD (Berenbaum et al 1986).

An exciting development has been to administer a pro-drug to generate endogenous porphyrin. 5-aminolaevulinic acid (ALA) (Deprenyl-USA - DUSA, Toronto, Canada) is a pro-drug, which can be given orally, intravenously or topically. It forms part of the haem biosynthetic pathway and is converted to the active photosensitiser protoporphyrin IX (Kennedy and Pottier 1992). The absorption of the porphyrins is, however, weak in the red range with a small peak at 630 nm. ALA is gaining an increasingly important role in PDT and is the sensitiser used in the experimental work of this thesis.

5.6.2 Chlorins.

Chlorins have a strong absorption in the red region. m-THPC (Scotia QuantaNova plc, Guildford, UK) is at an advanced stage of clinical trial directed at advanced oral cancers. These compounds tend to have peak absorption spectra in the range 650-700 nm (Bonnett 1995). The chlorins and bacteriochlorins are produced by progressive reduction of the porphyrins.

5.6.3 Phthalocyanines.

These compounds, again, have a strong absorption in the red region. The zinc derivative is biologically active (Novartis (Ciba-Geigy), Basel, Switzerland), but because of the extreme hydrophobic nature of these compounds, a liposomal delivery agent is required. A peak absorption spectra for these compounds is in the range 670-750 nm. The aluminium derivative is soluble in water and has considerable potential as a photosensitiser.

5.7 Light delivery.

The peak absorption of the porphyrins is in the blue range, however endogenous substances such as haemoglobin also absorb strongly in this part of the spectrum. This results in very little light penetration in this range. At wavelengths above 600nm the haemoglobin absorption spectrum falls considerably, and although the absorption of most photosensitisers is not maximal, it is this range that is generally chosen for PDT. PPIX, the active metabolite of ALA has a red absorption maxima at 635nm which is almost 35 times less than the blue absorption maxima at 405nm (Kimel et al 1989). Also the longer the wavelength the better the tissue penetration.

At a wavelength between 600 and 800 nm, and dependant on the tissue, approximately 30% of incident light is reflected in the surface layers (Svaasand et al 1989). The photons that are propagated are then multiply scattered before being absorbed. After a distance of 1-2 mm these multiple scattering events produce near isotropic light distributions in most tissues (Wyss et al 1995).

5.8 Light penetration in tissues.

Light penetration in tissues decreases exponentially with distance because of scattering and absorption. The penetration depth (mm) is defined as the distance corresponding to a decrease in the optical fluence rate by a factor of $1/e$ (where $e = 2.718$) (Svaasand 1984).

The fluence rate is the quantity of photons that pass through a defined area per second (mW/cm^2).

The average light penetration in tissues is about 1-3 mm at a light wavelength of 630 nm and 2-6 mm at 800 nm. Therefore if the optical penetration of a tissue is 3mm, the fluence rate at a depth of 3mm will be 37% of its rate at the surface. If the distance is doubled to 6mm the fluence rate will be $1/e^2$ or about 10%. The optical penetration depth of a tissue is a unique parameter of that tissue.

In a hollow organ such as the uterus where a light delivery system is placed within the uterine cavity, the fluence rate may be increased by a factor of 5 or 6 due to the additional internal reflection of light by the surrounding tissue walls (Wyss et al 1995).

5.9 The optical dose.

This is defined as the irradiation (W/cm^2) multiplied by the exposure time (seconds). The optical dose (J/cm^2) decreases with depth into the tissue in a similar way to the fluence rate. Altering the incident power or altering the exposure time can alter the optical dose.

Previous work has shown that endometrial destruction using ALA-PDT, in rabbits, is extremely successful. When the technique is transferred to humans it is much less successful. This is mainly due to the fact that the human endometrium is much thicker than the animal model.

5.10 Summary.

Studies to date have shown that ALA is a safe drug to use. Peak PPIX fluorescence levels appear to be seen between 3 and 6 hours after the administration of ALA with a degree of variability between species. Certainly the variability of PPIX levels is dependant on oestrogen (and therefore menstrual cycle).

ALA-induced PDT destruction of the endometrium using light at 630nm is very effective in a small animal model. The success of this modality for human endometrial ablation has not been forthcoming (Gannon 1998). So little data has been published in humans that is difficult to know how to alter parameters to enable a more consistent effect. Several variables may be apparent in the human. First the endometrium is much thicker than the animal model. Light distribution in the triangular-shaped cavity may be a problem.

SECTION TWO

EXPERIMENTATION.

CHAPTER 6: AIMS AND OBJECTIVES.

The review of non-hysterectomy treatments for menorrhagia has shown that none is entirely satisfactory. The use of ALA-induced PDT for the treatment of menorrhagia in women is particularly attractive because the principle is simple and the drug has been shown to be safe. The administration of the drug and the treatment itself has the potential to be performed without the need for a general anaesthetic and can therefore be thought of as an “ambulatory treatment”. Previous animal work has been very encouraging with ALA-induced PDT. The human, clinical, work has so far been disappointing with very poor success (Gannon et al 1998).

We have a technique for enhancing ALA-PDT of the endometrium in women that has not been tried previously, using the iron-chelator CP94.

The objectives of this thesis, therefore, are:

- i. to assess whether using topical CP94 in combination with ALA can maximise levels of PPIX in the endometrium;
- ii. to assess different preparations of ALA, using different solute mediums and different levels of pH;
- iii. to treat normal rabbit endometrium with PDT using ALA either alone or in combination with CP94 to find the conditions under which endometrial ablation is most complete and most long-lasting;
- iv. to assess the distribution of light in the human uterine specimens from hysterectomies with a range of light delivery

devices to find which gives the most even distribution of light to all areas of the endometrium, and

- v. to propose how these results might be taken forward in clinical studies.
- vi. to put PDT in context with other modalities for the treatment of menorrhagia.

CHAPTER 7: MATERIALS AND METHODS.

7.1 Introduction.

The endometrium has a phenomenal regenerating capacity in both humans and other mammals (Ferenezy 1977; McLennan and Rydell 1965; Schenker et al 1971). To prevent regeneration, endometrial destruction by photodynamic therapy (PDT) must extend within the endometrium to include the deep glandular tissue. To perform successful PDT to the endometrium resulting in lasting endometrial destruction, drug concentrations should be as high as possible throughout the endometrium. Higher drug concentrations will then result in an increased PDT effect, assuming that the light dose is above the threshold value. Systemic administration limits the endometrial drug concentration because of side effects. As a result topical administration with an enhancer would appear to be the best strategy.

We were interested in optimising human endometrial destruction, yet had to show the improvements in the animal model. Several other workers had shown PDT in the animal model to be highly effective (Wyss et al 1992; Yang et al 1993; Fehr et al 1996; Wyss et al 1996). Showing a difference in the PDT effect was, therefore, potentially difficult. Rather fortuitously the laser that we used for ALA-PDT was a commercially available Diode Laser (Diomed Ltd, Cambridge, UK). It was supplied with a quoted output of 630nm by the company. When we tested this output we measured it at 628nm (+/- 1 nm) (figure 7.1).

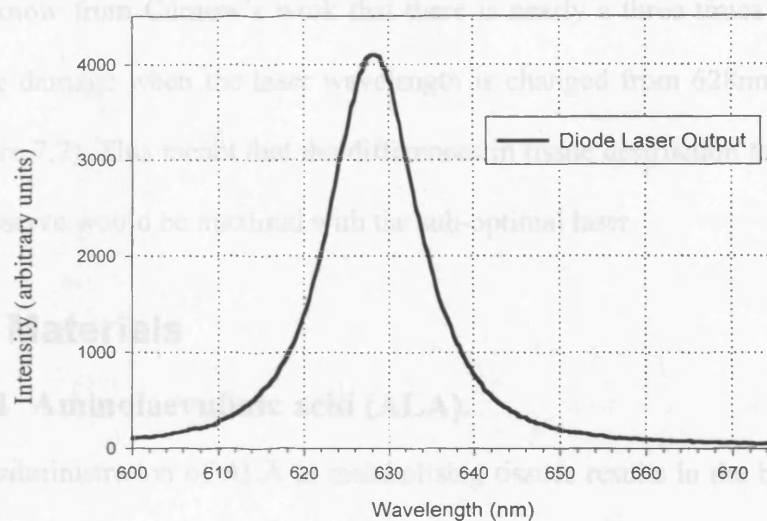


Figure 7.1: Measured wavelength of LASER output.

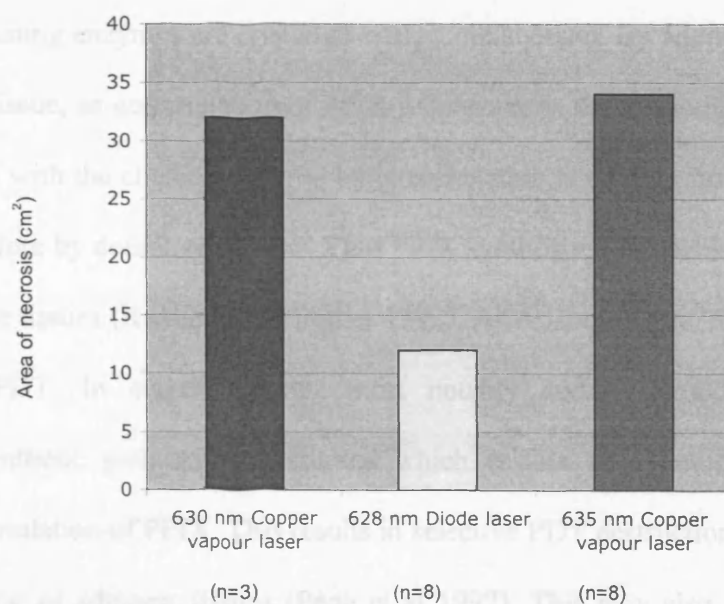


Figure 7.2: Area of necrosis produced by different lasers after PDT to the bowel mucosa.

We know from Curnow's work that there is nearly a three times increase in tissue damage when the laser wavelength is changed from 628nm to 635nm (figure 7.2). This meant that the differences in tissue destruction that we were to observe would be maximal with the sub-optimal laser.

7.2 Materials

7.2.1 Aminolaevulinic acid (ALA).

The administration of ALA to metabolising tissues results in the biosynthesis of protoporphyrin IX (PPIX). These tissues where there is an accumulation of PPIX will fluoresce at 670nm (red) when exposed to and excited by light at 635nm.

PPIX is the direct precursor of haem in the haem biosynthetic pathway (figure 7.3). All nucleated cells are able to synthesise haem. Also a number of haem-containing enzymes are crucial in energy metabolism. By adding excess ALA to a tissue, an accumulation of PPIX will occur; as the metabolism of PPIX to haem with the chelation of iron by ferrochelatase is the rate-limiting step, and therefore by definition is slow. Thus PPIX synthesised *in vivo* is used for PDT by the tissues (Kennedy and Pottier 1992). ALA is, therefore, a pro-drug used for PDT. In certain tissues, most notably certain tumours, the haem biosynthetic pathway is disrupted which results in a naturally occurring accumulation of PPIX. This results in selective PDT destruction, with relative sparing of adjacent tissues (Peng et al 1997). This may also be utilised for photodiagnosis.

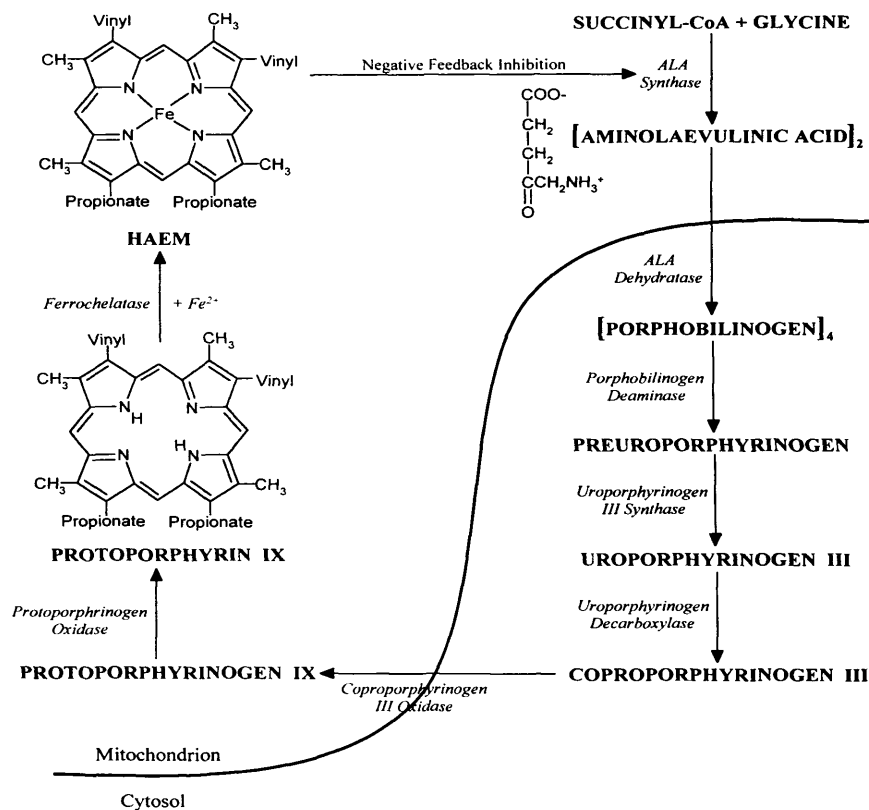


Figure 7.3: Schematic diagram of the haem biosynthesis pathway.

The committed step in the synthesis of haem is the condensation of glycine and succinyl-CoA. The enzyme responsible for this is ALA synthase. It is located within the mitochondria, and ALA is exported from the mitochondrion to the cytosol. Exogenous ALA, which rapidly enters cells is therefore easily converted in the cytosol to coproporphyrinogen III which is then transported into the mitochondrion, to form PPIX (Jordan 1991). An increase in the haem concentration within the mitochondrion has a negative feedback effect on ALA synthase, thus reducing further production of haem. However, by the

exogenous administration of ALA, this first major control point in the cycle is bypassed; there is then a build up of substrate causing a maximal rate of operation of all enzymes in the cycle. PPIX accumulates as the enzyme ferrochelatase is working relatively slowly. PPIX itself is poorly soluble and therefore it is not practical to simply administer this to tissues. The administration of other precursors is also limited by solubility and costs (Kennedy and Pottier 1992).

ALA is highly water-soluble and can be administered systemically, orally, topically or intravenously. ALA in aqueous solution is extremely acidic (pH 1 - 1.5), but when buffered to a physiological pH (pH 6 - 7) is highly unstable. When buffered to pH 4 - 5 it is reasonably stable (Elfsson et al 1998).

ALA is thought to enter cells by active transport, but passive diffusion may also occur. Once inside cells, the primary sites of damage on administration of light are those where PPIX is produced - i.e. the mitochondria. It may also be that damage occurs without light as extremely high levels of ALA and PPIX may disrupt respiration and normal cell function (Peng et al 1997).

Unlike other photosensitisers, the PDT effect is maximum within a few hours of administration of ALA. Photofrin, by contrast is administered 48 hours before PDT (Stewart et al 1998). Side-effects with ALA are also much less. The most notable is skin sensitivity, lasting 24-48 hours or so. This is also reduced by topical administration.

ALA esters are currently being investigated as a way of improving treatment. The esters are more lipophilic and therefore enter cells more rapidly (Peng et

al 1997). Other methods are used to improve penetration either with azone (Steiner et al 1996), or DMSO (Peng et al 1997).

7.2.2 1,2-diethyl-3-hydroxypyridin-4-one (CP94).

The hydroxypyridinones are a relatively new group of iron chelators. They are active orally and enter the intracellular iron pools rapidly, being of a low molecular weight and of neutral charge (Hoyes et al 1993). These were designed to supercede desferrioxamine for the treatment of iron overload states. They are well suited to enhancing ALA-induced PDT, as they are of a lower molecular weight and have greater lipophilicity than desferrioxamine (Brittenham 1992).

CP94 has been used orally in patients with little toxicity, providing effective iron mobilisation (Brittenham 1992). It is rapidly absorbed orally, and enters all cells by simple diffusion (Hoyes et al 1993). It is excreted in the urine after glucoronidation, something that cannot happen in rats, but can in rabbits (Porter 1993). It is bidentate compared to desferrioxamine, which is hexadentate, and has a much higher affinity for iron, binding in the ratio of 3:1 compared with 1:1 with desferrioxamine (Hershko et al 1991).

Little work has been carried out on CP94 and ALA-induced PDT. It has been shown to double the PPIX fluorescence in the urothelium of the rat bladder when used in combination with ALA (Chang et al 1997). It has also been used in rat colon to increase the PDT effect (Curnow et al 1998). Previous work with ALA-induced PDT has used CP94 intravenously.

CP94 will inhibit the final pathway in the conversion of PPIX to haem by removing iron (Fe^{2+}) from the pathway. There is thus more PPIX available for

PDT. Desferrioxamine, and ethylenediamine tetraacetic acid (EDTA) have been investigated as enhancers of ALA PDT (Chang et al 1997). EDTA has poor penetration into cells. Desferrioxamine is therefore a better enhancer of ALA-induced PDT (Berg 1996). CP94 was developed to supercede desferrioxamine in the management of iron overload states (Porter 1996).

7.3 Endometrial photosensitisation.

We undertook an initial series of experiments to investigate the pharmacokinetics of ALA in the normal rabbit uterus, used alone and in combination with 1,2-diethyl-3-hydroxypyridin-4-one (CP94). These experiments were to investigate whether the topical co-administration of CP94 would have any effect on the absorption of ALA. There is considerable experience using the rabbit model with a number of different photosensitisers and ALA especially (Yang et al 1993; Wyss et al 1994; Fehr et al 1996; Wyss et al 1996). The pharmacokinetics of the hydroxypyridinone 1,2-diethyl-3-hydroxypyridin-4-one (CP94) has not been studied in this animal model.

The inherent fluorescent properties of PPIX were used to identify, localise, and quantify the levels of photosensitiser within the different layers of the rabbit uterus, using the fluorescence microscope. The similar structure of PPIX and haem are noted in figure 7.4. It was necessary to confirm other workers' findings about time of peak fluorescence in order to determine optimal treatment parameters prior to commencing the photodynamic therapy studies.

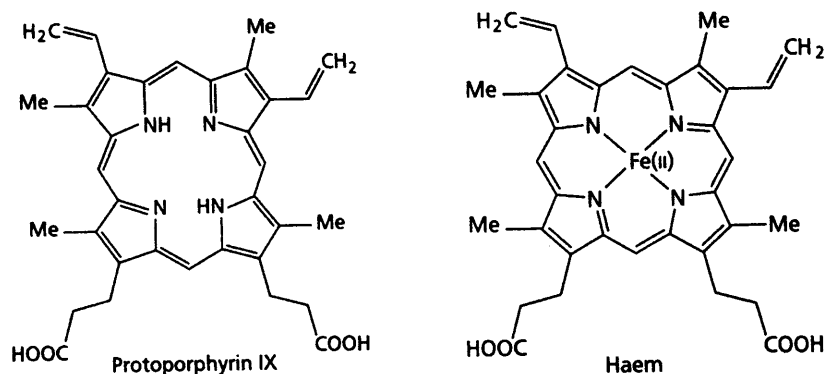


Figure 7.4: Chemically similar structures of PPIX and Haem

ALA absorption has not been studied at different pH. We undertook a study to compare the pharmacokinetics of buffered and unbuffered solutions of ALA used alone and in combination with CP94.

Once the optimal conditions were identified, we performed PDT with both ALA alone and in combination to investigate whether there is a difference in PDT effect by the addition of CP94. In order to prevent regeneration we also undertook long-term studies after PDT to assess the permanency of the treatment.

Within the study period several interesting observations were made, and so new experiments were undertaken. An example is the photobleaching experiment, which was not in our initial planned series of experiments. To ease the experimental progression it will be dealt with first, and not in chronological order.

7.4 Animal model.

In women the majority of the genital tract develops from the Müllerian or paramesonephric ducts, extending from the genital ridge where the gonads develop, to the urogenital sinus. The lower ends of the two ducts fuse in the fallopian tubes, in close proximity to the ovaries. There is thus a single uterus with a midline cavity and two fallopian tubes (figure 7.5).

The thickness of the endometrium is 10 - 20 mm depending on the cycle. The only mammals with a similar uterine structure are primates. Primarily because of ethical reasons, as well as the number of animals that we would require and therefore the cost, it is inappropriate to use primates. The rat model was also deemed inappropriate because the rat uterus is made of two uterine horns, measuring 1-1.5 cm in length with an extremely thin wall. Instrumentation would therefore be difficult, and as cannulation of the structure was required, trauma may have had a profound influence on the experimental validity.

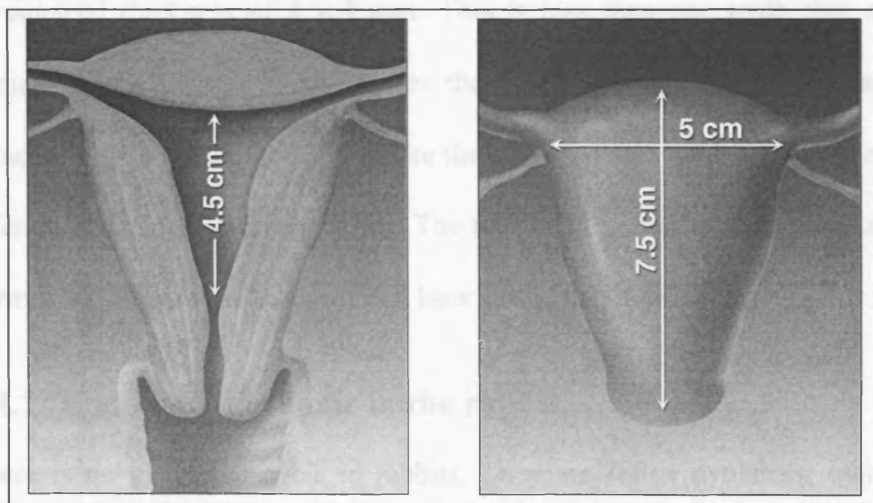


Figure 7.5: Human uterus

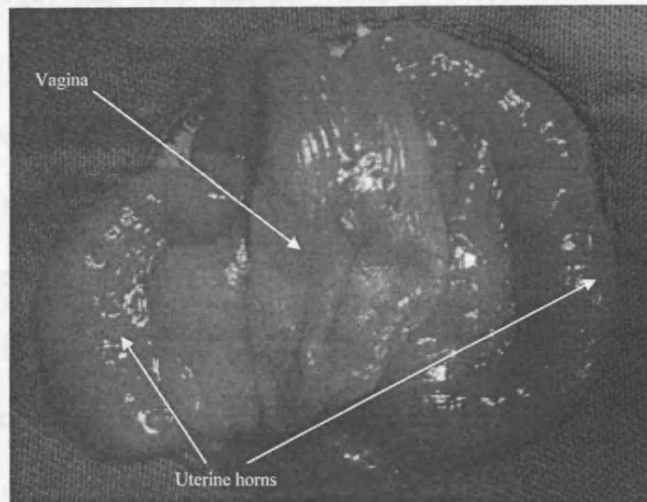


Figure 7.6: Rabbit uterus exposed at laparotomy

The rabbit uterus is similar to the rat, in that it has a duplex structure consisting of two cervixes and two uterine horns of 7 - 10 cm in length (figure 7.6). The thickness of the endometrium by contrast is 2 - 6 mm with a myometrial thickness of 1 - 3 mm. This is less than one tenth that of the human uterus. This does mean that the potential for collateral damage to adjacent organs would be high, unlike the human where the thick myometrium offers a considerable safety buffer. The tubular structure of the rabbit uterine horns also allows us to use standard laser fibres for illumination.

7.4.1 Endometrial repair in the rabbit.

There is no menstrual cycle in rabbits. They are reflex ovulators; ovulating after copulation or an artificially-induced LH surge. Menstruation can be induced by curettage of the cavity (Schenker et al 1971). The regeneration seen after curettage (Schenker et al 1971) or cryotherapy (Schenker and

Polishuk 1972) has been studied. There is more extensive damage with cryotherapy. Early regeneration occurs from the basal crypts of the endometrium and is seen within 3 and 12 hours respectively. The primary cells are flattened and thus have an extensive coverage within a short period of time. Full coverage of the denuded endometrium occurred in the curettage group within 48 hours. With maturation of these cells, they became more cuboidal and eventually columnar. Internally the rabbit endometrium is arranged with finger-like projections (villi) - similar to that found within the fallopian tubes of the human. This poses a problem of how to adequately illuminate the cavity with PDT. In order to adequately illuminate the deep crypts, distension should be employed. High-pressure distension would flatten the villi allowing complete illumination. However this pressure would reduce the blood supply and therefore the oxygenation, hence reducing the photodynamic effect. It was therefore decided to use the standard diffuser fibre, to keep the experimentation identical, but with the risk of inadequate illumination to the crypts.

7.5 Methods.

7.5.1 Chemicals.

The Aminolaevulinic acid (ALA) powder (ALA HCl, 99% purity, DUSA Pharmaceuticals Inc., New York) was dissolved in saline. It was administered into the uterine horns of the rabbits with a concentration of 400mg/ml. The 1,2-diethyl-3-hydroxypyridi-4-one (CP94) was synthesised and donated in powder form from the Pharmacy department at Kings College London (95% purity). This was dissolved with the ALA using saline. The resultant solution was ALA 400 mg/ml and CP94 100mg/ml.

The buffered ALA solution was prepared by dissolving 1000mg of the ALA powder in saline (0.9%). This was buffered by the addition of phosphate solution made of 143mg of disodium hydrogen phosphate and 11.4mg of potassium dihydrogen phosphate in 10ml of water (23% disodium hydrogen orthophosphate; 1.9% potassium dihydrogen orthophosphate), making a solution with a pH of 4.5-5.0. The solution was made up with additional saline to 2.5 ml. The resultant solution was of 400mg / ml ALA. The ALA and CP94 combined buffered solution was prepared by adding 250 mg of CP94 powder with 1000 mg of the ALA powder, the resultant solution was buffered as above. Saline was added to make the solution up to 2.5 ml. The resultant solution was of 400mg / ml ALA and 100 mg/ml CP94.

The unbuffered solutions were made up to the same concentrations using saline only but no buffer was used. The pH of the resultant solution was 1.5-2.0.

Solutions were made up immediately prior to use, as buffered ALA solutions are known to be unstable (Chang et al 1997). Commercially available phosphate buffer (PBS) has insufficient buffering capacity for the ALA solutions described. Separate syringes and needles were used for each solution during the experiment.

7.5.2 Laser.

The laser we used was a commercially available Diode Laser (Diomed Ltd, Cambridge, UK) (figure 7.7). The laser output was measured at 628 nm (\pm 1 nm) as described earlier in this chapter.

7.5.3 Laser diffuser fibres.

The laser diffuser fibres were radially emitting plastic fibres (cylindrical diffusers) which can be used for intraluminal PDT. The fibre has an external diameter of 0.94 mm, is highly flexible with a minimum bend radius of 20 mm. The core diameter is 0.5mm.



Figure 7.7: Diomed diode laser, with diffuser fibres and beam splitting box

The light emitted is homogeneous all along the diffuser's tip, and not forward pitched, allowing a more controlled light dose in a hollow organ. The diffuser tip length was 40 mm.

7.5.4 Animal handling.

The New Zealand white rabbit was used. It is albino and has prominent ear veins, which are easily identified and cannulated, with little discomfort for the animal. Sexually mature females of around eight months of age were used with a body weight of 2.9 - 4.0 kg. Animals were supplied by Charles River UK Ltd, (Margate, Kent). They are resistant to disease when held in sanitary conditions, and are an unlikely vector to man. Proper handling of the animals is necessary to prevent animal or human injury. The animals were housed in specially designed cages and kept in a controlled environment and given free access to food and water, for at least seven days prior to experimentation. All experiments were approved by the Home Office (UK) and were carried out under licence.

7.5.5 Anaesthesia and drug administration.

Anaesthesia was used to render the animals immobile, insensitive to pain and to allow the surgery to occur. The rabbits were given a general anaesthetic by a single, trained, operator. Adequate analgesia was also required post-operatively. Advice and assistance was given by the department of Biological Sciences at University College London.

Animals were given a pre-medication of Hypnorm (Fentanyl Citrate and Fluanazone) (Janssen Pharmaceuticals Ltd., Oxford, UK) 15 minutes before induction of anaesthesia. Anaesthesia was induced with Diazepam (Phoenix

Pharmaceuticals Ltd., Gloucester, UK) and maintained with additional diazepam as required. Buprenorphine hydrochloride (Reckitt and Coleman Ltd., Hull, UK) was used for post-operative analgesia.

Regular observations of the rabbits' pulse were made. An estimation of oxygen saturation was made using a pulse oximeter.

7.5.6 Surgical procedures.

Post-mortem examination was made of animals used and killed in experiments carried out by other workers at the University in order to become familiar with the anatomy of the rabbit. All instruments were cleaned and sterilised by autoclave. Animals were anaesthetised and shaved using a small electric shearer. The shaved area was cleaned with alcoholic chlorhexadine and allowed to dry for two minutes. Sterile drapes were used to minimise contamination from adjacent fur (figure 7.8).

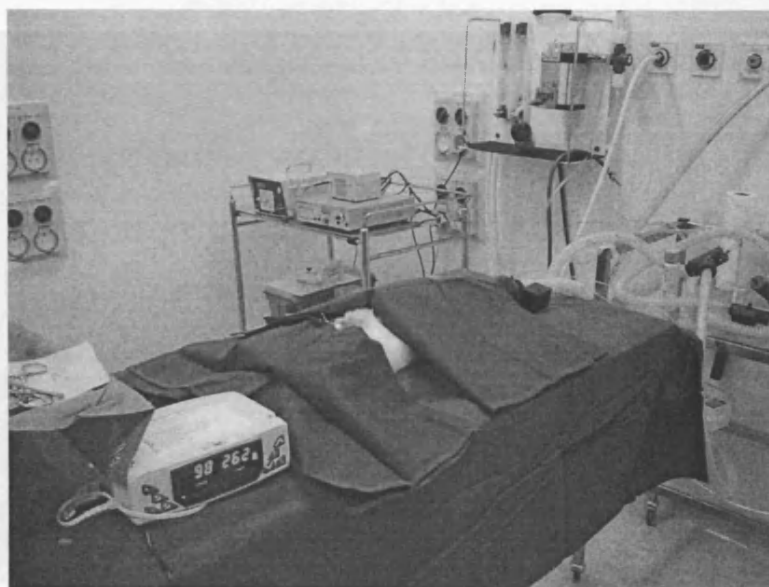


Figure 7.8: Rabbit anaesthetised in theatre.

Fine instruments were used, a scalpel and disposable blades, a needle holder, toothed forceps, fine scissors and suture scissors. A 3 - 4 cm low midline incision was made in the skin. The peritoneal cavity was entered by careful elevation and cutting with scissors to prevent bowel injury. The uterine horns were identified behind the bladder and with minimal handling, exteriorised. In order to illuminate the uterine horns, a laparotomy was performed under general anaesthesia so that a dam diffuser fibre could be inserted into each exteriorised horn. Silk ties were applied to both the proximal and the distal ends of the uterine horns to prevent spill from one horn to the other, an extra suture was placed on the horn with the CP94 to confirm identity of the two horns later. The bladder was emptied if required using a fine gauge needle and syringe.

7.5.7 Drug instillation.

The solution containing ALA alone was given to the left horn by injection through the ante mesenteric border approximately 0.5cm distal to the bifurcation, using 25G needles (figure 7.9). The total volume instilled was 0.5ml (200mg ALA). The ALA / CP94 combination was given to the other

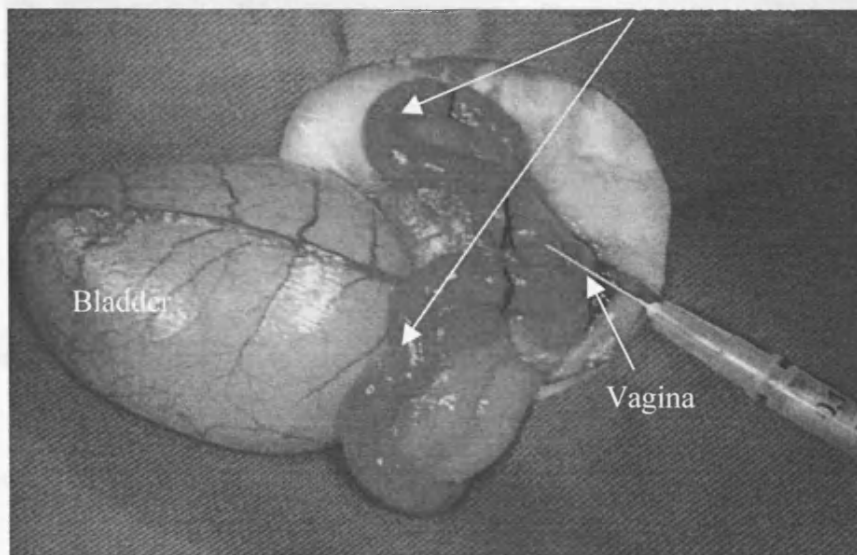


Figure 7.9: Drug instillation into the rabbit uterine horns.

horn in a similar manner, the total volume being 0.5ml (200mg ALA, 50mg CP94). Separate syringes and needles were used for the two solutions. The abdomen was closed in two layers using continuous silk sutures.

7.5.8 Laser illumination.

In order to illuminate the uterine horns, a laparotomy was performed under general anaesthetic so that a 4cm diffuser fibre could be inserted into each exteriorised horn.

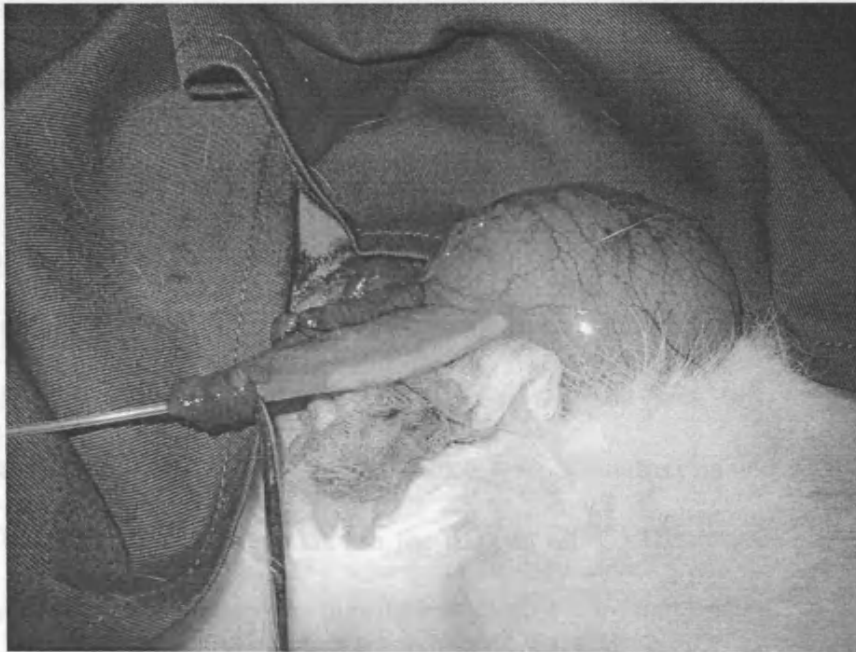


Figure 7.10: Laser fibre being inserted into uterine horn.

They were carefully placed through the distal end of the uterine horn and fed through the proximal horn and partially through the cervix (figure 7.10). A

beam splitter facilitated the use of two fibres simultaneously (figure 7.7). A total light dose of 100J/cm fibre (output power 100mW/cm fibre) was delivered to each using a 628nm diode laser (Diomed, Cambridge, UK). The animals were then recovered.

7.5.9 Tissue retrieval.

The animals were killed using high doses of intravenous phenobarbitone.

Tissue specimens removed for fluorescence studies were immediately frozen by submerging in isopentane (2-methylbutane) pre-cooled in liquid nitrogen. These snap frozen specimens were stored in liquid nitrogen until sectioned. Tissue specimens for histological analysis were submerged in formalin.

7.5.10 Fluorescence microscopy studies.

Tissue blocks were mounted on OCT medium (Tissue trek II embedding compound, BDH) and ten μm thick sections were cut from each block using a Cryocut E microtome (Reichert-Jung). One section was left frozen and unstained. An adjacent section was stained with haematoxylin and eosin (H & E). The frozen slides were kept in the dark at -20°C . These were allowed to thaw just before fluorescence microscopy studies were undertaken.

Structures were identified with phase contrast microscopy. Fluorescence images were taken with a slow-scan cooled charged-coupled device (CCD) camera (Wright Instruments, Enfield, London, UK) with a 400 x 600 pixel resolution. Fluorescence was excited using an 8 mW helium-neon laser (632.8 nm). It was decided to use the helium-neon laser as an excitation source for two reasons. First the laser is cheap, easy to use and maintain and gives a predictable output. Second, when exciting at this wavelength tissue

autofluorescence is low. Fluorescence was detected between 665 and 710 nm using bandpass and longpass filters.

Quantitative microfluorimetry was carried out in a dark room. An inverted microscope (Olympus IMT-2) with epifluorescence and phase-contrast attachments was used (figure 7.11).

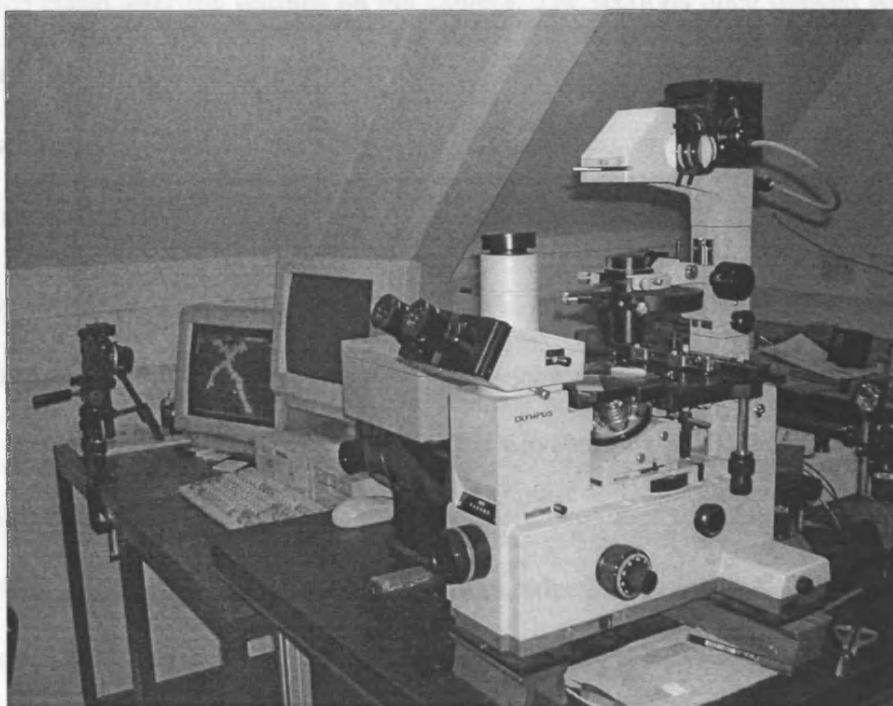


Figure 7.11: Fluorescence microscope and detection and assimilation hardware.

An 8 mW HeNe laser was used to excite fluorescence. The beam was delivered through a liquid light guide, through a 10 nm band-pass filter centred at 633 nm to remove extraneous light, and onto the dichroic mirror (Omega Optical Inc.) for epifluorescence studies. The fluorescence detection range was from 665 nm to 700 nm centred at 675 nm by band-pass and long-

pass filters. The long-pass filter (>665 nm) removed scattered excitation light. The low fluorescence yield from the HeNe excitation was circumvented by using a highly sensitive slow scan CCD camera. The camera is normally used for astronomy. Because of the nature of the sensitivity, it had to be pre-cooled with liquid nitrogen prior to use. This cooling lowered the electronic 'noise' picked up by the camera. The camera was attached to a personal computer which could alter the settings on the camera. The sections once thawed were examined with phase contrast microscopy and when a representative section was identified, background lights were extinguished, and the laser and CCD camera activated.

The recorded signal was processed on a PC and a false colour-coded image projected depicting the mean fluorescence signal counts per pixel (20 photons per count; quantum efficiency = 0.5 at this wavelength). A false colour-coded image of the fluorescence signal was therefore produced and the fluorescence intensity in the superficial endometrium, deep endometrial glands and myometrium was quantified digitally, by averaging over specified areas. All measurements were corrected for background autofluorescence.

Fluorescence emission spectra were also recorded from separate representative frozen sections to confirm that the fluorescence obtained was produced by PPIX and no other fluorescent compound. This was carried out by connecting a spectrograph (Multispec 1/8 m, Oriel Instruments, Connecticut, USA) with a slow-scan cooled CCD camera (Wright Instruments) via a fibre-optic bundle positioned directly above the opened specimen (endometrium uppermost) to an inverted microscope. Fluorescence was excited using a 1mW helium-neon laser at 543nm, and the emission spectra were recorded over 615-735nm with

a 1nm resolution. Scattered excitation light was suppressed with a RG590 filter. The epifluorescence excitation was confined to a 100 μ m spot, aligned to the area of interest with a phase contrast microscope. There were no photobleaching effects noted; integration times were 10 seconds.

7.5.11 Histological studies.

Histological assessments were carried out with the assistance of a consultant histopathologist from University College London Hospitals. She specialised in (human) gynaecological pathology. Measurements were made using a light microscope at magnifications of x16 and x32 using 0.1mm graticules. The thicknesses of the endometrium, including the maximal and minimal thickness of the endometrial folds were measured, and a ratio obtained. The stromal and the myometrial thickness were measured. The number of glands in the superficial and deep endometrium was noted. For each specimen four separate measurements were made. All the measurements were averaged and a score given of zero, one, two or three.

7.5.12 Scoring system.

Previous studies assessing damage to the endometrium have used very subjective measurements or merely descriptive terms (Wyss et al 1994; Wyss et al 1996). In order to measure destruction more objectively we developed a scoring system based on the measurements made from the thickness of the uterine wall, endometrium and stroma. We included the number of superficial glands seen in a particular length of endometrium and also the number of deep glands seen per unit area. These measurements were tabulated and a score obtained. The scoring system was modified slightly and tested with three

CHAPTER 8: PHARMACOKINETIC STUDIES

8.1 Experiment 1: Light bleaching

8.1.1 Introduction.

This small experiment was undertaken after seeing the methods used to obtain the cryosections. We were exceptionally careful to avoid light exposure of the harvested uterine specimens, and once we obtained the cryosections, again we were careful to avoid light exposure prior to measuring fluorescence. However, the technicians who kindly cut the sections used high illumination during the preparation of the uterine specimens and cutting the cryosections. This was of concern and so a simple experiment was undertaken to investigate the effect of photobleaching on the fluorescence of the specimens.

8.1.2 Methodology.

Samples were taken from the uterine horns of four rabbits that had undergone the initial pharmacokinetics experiment. The ALA instilled was buffered to pH 4.5 - 5.0. The samples used were taken at four hours after administration of ALA. Two samples were used from each of the four animals. Eight slides were, therefore, were used to analyse fluorescence as outlined above. The slides were deliberately prepared by one of the senior technicians using very low light conditions.

The slides were viewed and a suitable area identified. Two fluorescence measurements were made on each side of this area at the '9 o'clock' and '3 o'clock' positions, from each slide. The slides were then placed under an angle-poise table light as used by the technicians cutting the sections. Every 5

minutes each slide had fluorescence measured from the same section of the specimen, and were replaced under the lamp. The experiment was concluded after 120 minutes.

There were therefore 48 readings from each slide. All the results at each time point were averaged and the medians calculated and the interquartile range. Statistical analysis was performed using the Mann-Witney U test. Significance was deemed to be reached with a p value <0.05.

8.1.3 Results.

The median fluorescence levels at each time point are plotted on the graph (figure 8.1.1). There was no significant difference between any of the times. Full results are displayed in appendix 3.

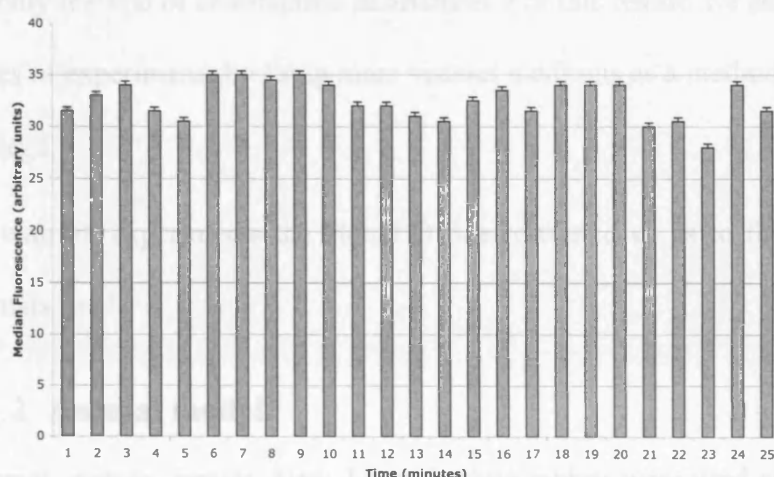


Figure 8.1.1 : Median fluorescence

8.2 Experiment 2: Pharmacokinetics in the rabbit uterus of topically applied ALA and CP94.

8.2.1 Introduction.

Although there is considerable experience of ALA absorption in the rabbit uterus (Wyss et al 1992; Yang et al 1993; Fehr et al 1996; Wyss et al 1996) there are no data regarding ALA and CP94 absorption. The first part of these series of experiments was using buffered solutions as used by other workers (Wyss et al 1992; Fehr et al 1996; Wyss et al 1996). The second set of experiments in this series used unbuffered solutions.

All these solutions have the same viscosity as water and therefore have the potential to simply spill out of the uterus unless prevented from doing so with some form of physical barrier. In our experiments the uterine horns were simply tied. For clinical practice we are trying to develop a highly user-friendly method of endometrial destruction. For this reason we concluded this series of experiments by using more viscous mediums as a method of reducing spillage.

As with all experiments the Home Office restricted us as to the number of animals used.

8.2.2 Animal model.

Normal, mature, female, New Zealand white rabbits were used as outlined in chapter 7. All experiments were approved by the Home Office and were carried out under licence.

8.2.3 Chemicals.

ALA and CP94 were supplied and used as outlined in chapter 7.

8.2.4 Procedures.

Animals were prepared as described earlier. The uterine horns were identified and with minimal handling, exteriorised. Silk ties were applied to both the proximal and the distal ends of the uterine horns to prevent spill from one horn to the other, and to identify the two horns later. The solution containing ALA alone was given to the left horn by injecting 0.5 ml through the antemesenteric border approximately 0.5cm distal to the bifurcation, using 25G needles. The solution containing the ALA / CP94 combination was given to the other horn in a similar manner, the total volume being 0.5ml (figure 8.2.1).

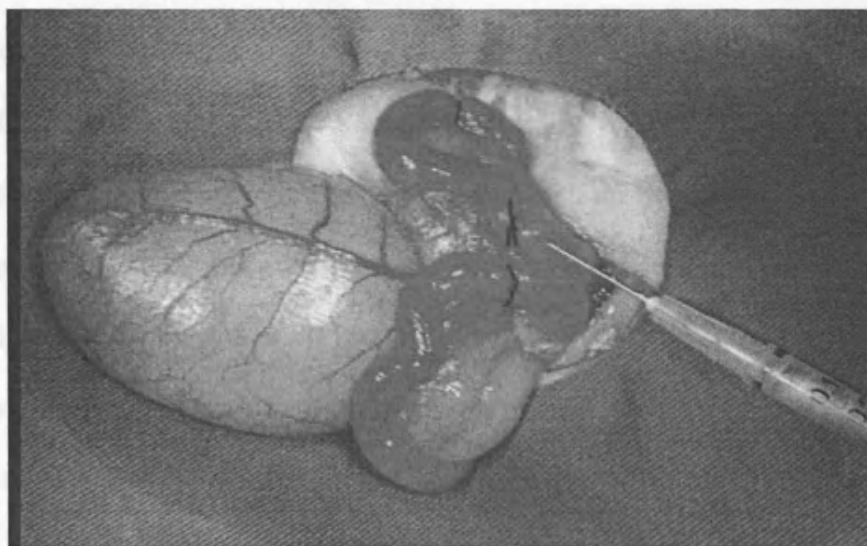


Figure 8.2.1: Solution being injected into rabbit uterine horn.

The animals were recovered and then killed at various times after the dosage (2-12 hours). Each uterine horn was excised and snap frozen in liquid nitrogen. Ten-micrometer thick cryosections were then prepared, with adjacent haematoxylin and eosin (H and E) staining. For the buffered solution experiment two animals were used at each time; 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours after administration. For the unbuffered experiment two animals were used at each time; 2, 4, 6, 7, 8, 10 and 12 hours after administration. There were 4 blank control animals.

The frozen sections were analysed by fluorescence microscopy. Fluorescence emission spectra were reported to confirm that the fluorescence observed in the imaging was produced by PPIX and no other fluorescent substance.

8.2.5 Fluorescence Studies.

Phase contrast microscopy with a slow-scan cooled charged-coupled device (CCD) camera was used to image and quantify fluorescence on the frozen sections. Fluorescence was excited using a helium-neon laser as described previously. Measurements were made from the superficial endometrial glands (adjacent to the lumen), the deep glands (adjacent to the junction with the myometrium) and from the myometrium of each section. Multiple sections were analysed from each horn. Two uterine horns, from different animals were used for each set of time parameters.

8.2.6 Results I: Buffered solutions.

No adverse effects were observed during or after administration of the drugs.

Full results are outlined in appendix 3.

8.2.6.1 *Fluorescence Spectroscopy.*

Normalised fluorescence spectra were obtained from the frozen sections from animals given ALA alone, the ALA / CP94 combination, and from untreated control animals. No differences were observed in the absorption spectra taken from each of the two treatment regimens. Maxima were seen at 632nm (+/- 2nm) in each case and these conform to the standard PPIX absorption spectra (figures 8.2.2 and 8.2.3).

8.2.6.2 *Tissue fluorescence quantification.*

Figure 8.2.4 shows a representative set of photographs of typical false colour-coded CCD images of the uterus with matched H & E stained histology seven hours after installation of ALA and CP94.

Figure 8.2.5 shows a set of photos of the uterus with matched H & E histology seven hours after instillation with ALA alone.

Figure 8.2.6 shows a representative photo of the fluorescence in the myometrium with an adjacent H & E.

Even to an untrained observer the fluorescence images show higher fluorescence using the ALA/CP94 combination than ALA alone. It is quite gratifying to see that even the deeper glands have high levels of fluorescence in both examples.

The low levels in the myometrium appear to confirm our hypothesis that there will be a good safety margin preventing extra-uterine damage.

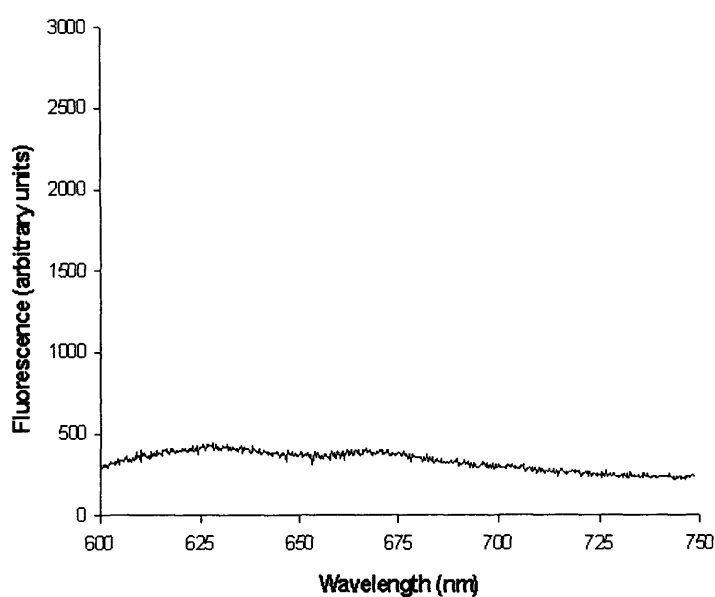


Figure 8.2.2: Fluorescence spectra from a normal untreated uterine horn.

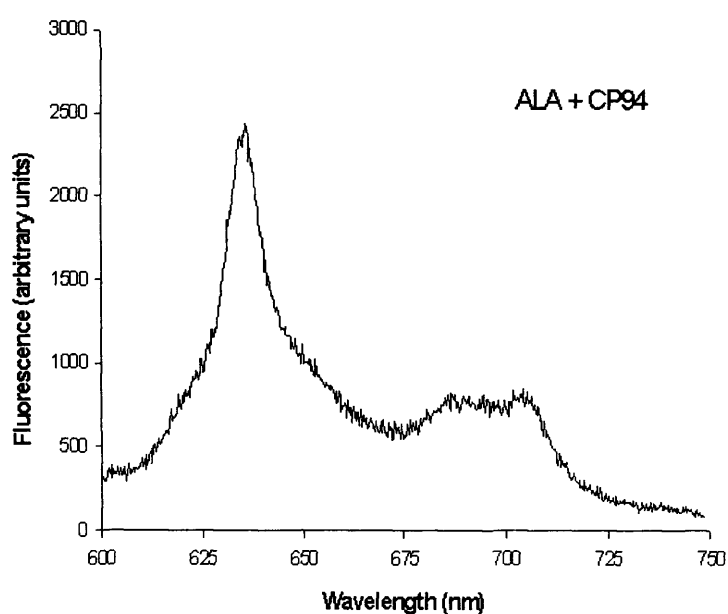


Figure 8.2.3: Fluorescence spectra from a normal uterine horn treated with ALA and CP94 with a peak at the correct wavelength for PPIX.

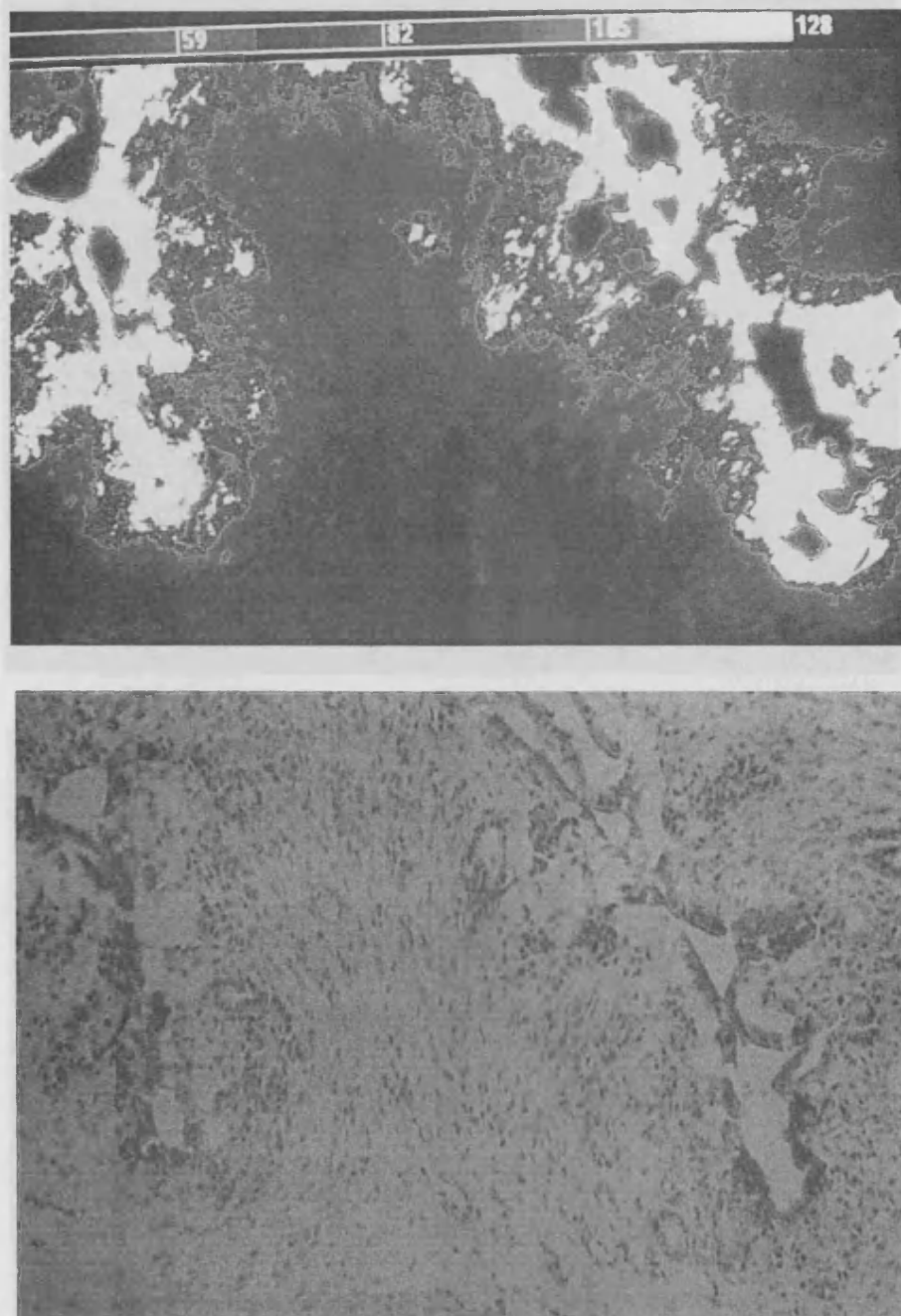


Figure 8.2.4: A set of photos showing the endometrial fluorescence with matched H & E histology seven hours after instillation with ALA and CP94.

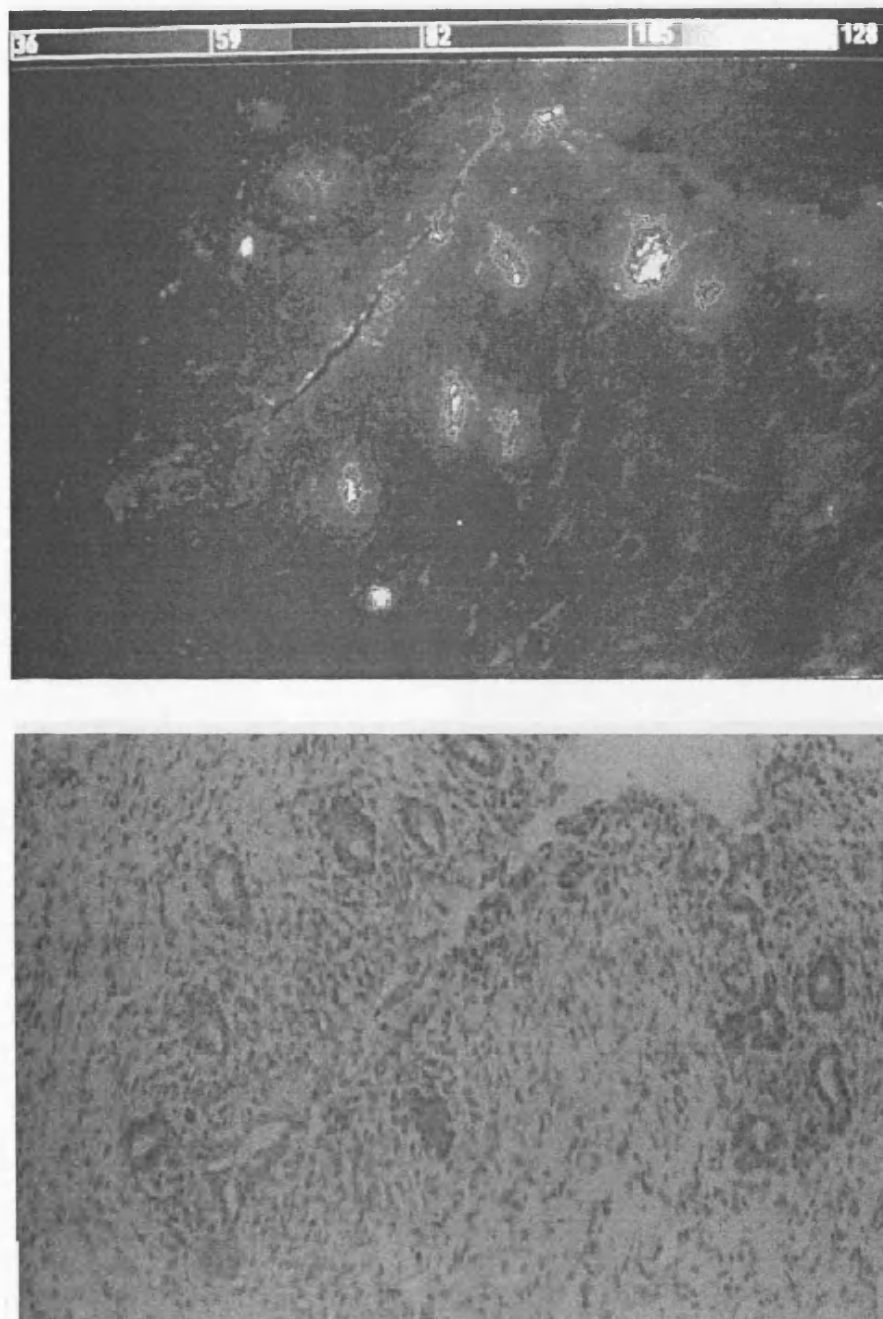


Figure 8.2.5: A set of photos showing the endometrial fluorescence with matched H & E histology seven hours after instillation with ALA alone.

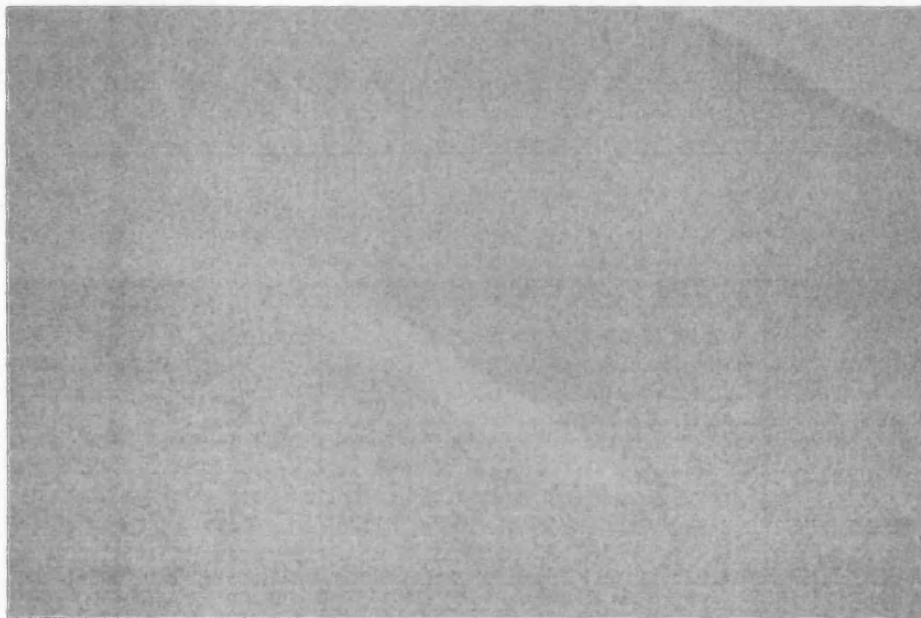
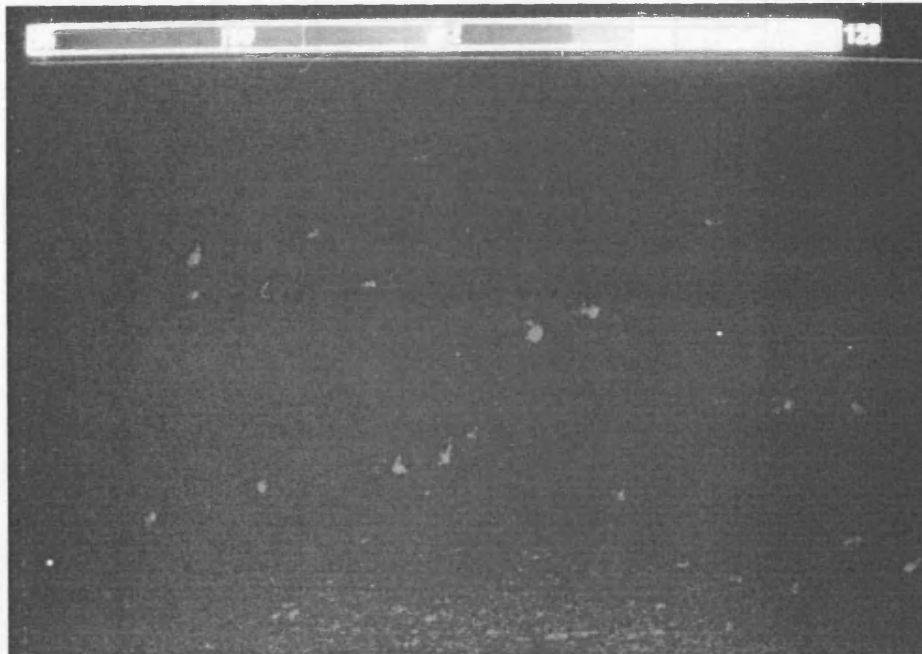


Figure 8.2.6 A set of photos of the myometrial fluorescence with matched H & E histology seven hours after instillation with ALA and CP94.

Figure 8.2.6. Fluorescence at the deep endometrial glands.

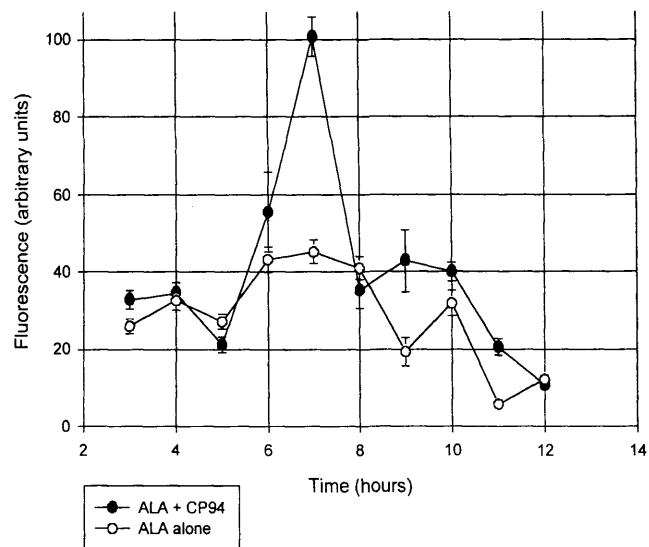


Figure 8.2.7: Fluorescence in the superficial endometrium

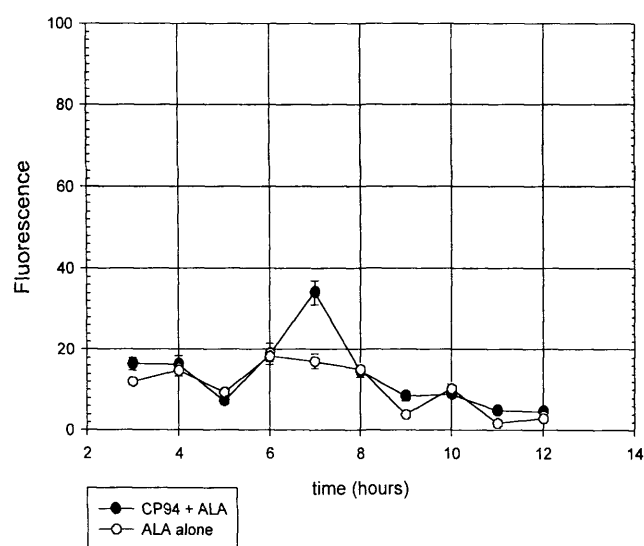


Figure 8.2.8: Fluorescence in the deep endometrial glands.

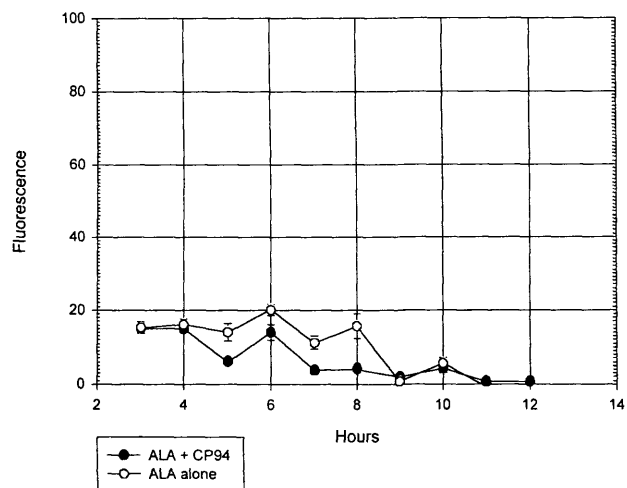


Figure 8.2.9: Fluorescence in the myometrium

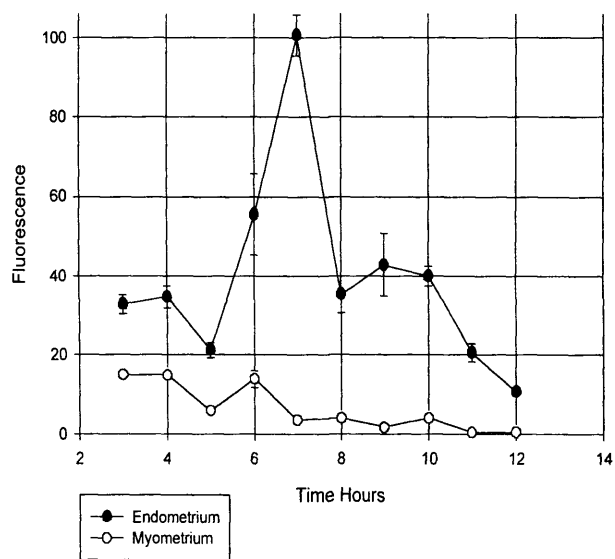


Figure 8.2.10: Comparison of superficial endometrium and myometrium using ALA and CP94.

Figure 8.2.7 compares the fluorescence levels using ALA alone and the combination of ALA and CP94 in the superficial endometrium. Figure 8.2.8 compares the same in the deep endometrial glands, and figure 8.2.9 compares fluorescence in the myometrium. els in the myometrium.

Figure 8.2.10 compares the superficial endometrial fluorescence with the myometrium using the combination of ALA and CP94.

Background autofluorescence has been subtracted from all measurements. Full results are tabulated in appendix 3.

Figure 8.2.11 shows how the fluorescence in the superficial endometrium, deep glands and myometrium varied with both time and drug combination.

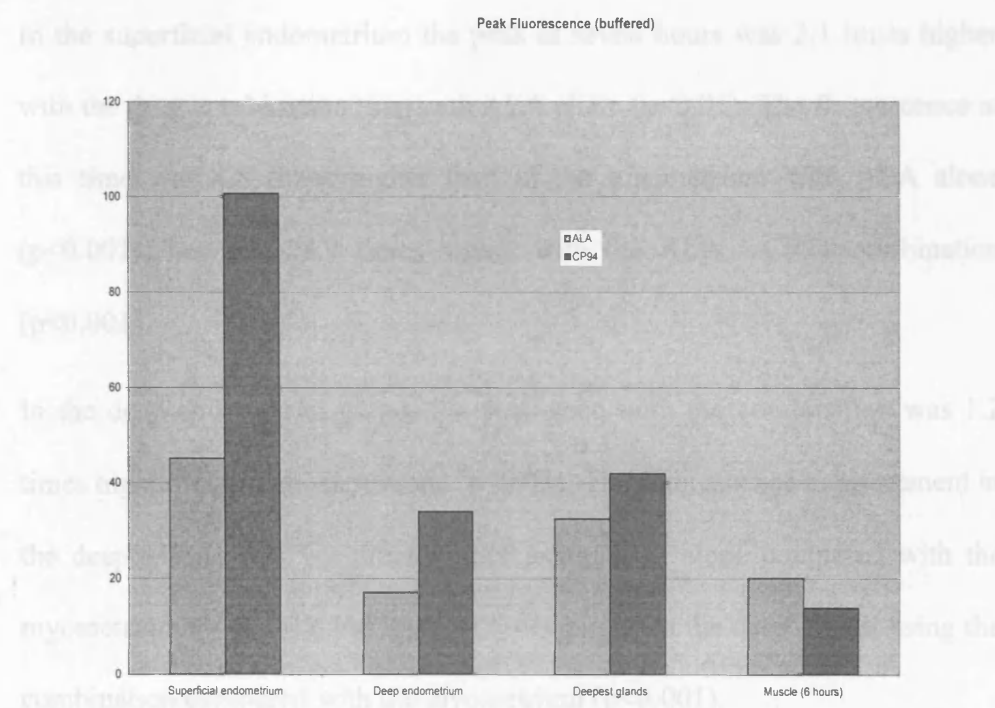


Figure 8.2.11: Simplified chart comparing ALA alone "ALA", with ALA + CP94 "CP94", seven hours after administration.

Peak fluorescence was observed in the superficial endometrium seven hours after sensitisation with ALA alone (median 48 arbitrary units - a.u.), or after the combination of ALA and CP94 (median 101 a.u.) ($p<0.01$).

Results from the deep glands at a peak of seven hours after sensitisation revealed median peak fluorescence with ALA alone of 18.5 a.u., and with the combination, 31.5 a.u. ($p<0.01$).

Median fluorescence in the myometrium at seven hours was 10 a.u. with ALA alone and 3.5 a.u. with the combination. However, the median fluorescence at six hours in the myometrium was significantly higher than the seven hour measurements at 20.1 a.u. with ALA alone and 13.9 a.u. using the combination ($p<0.01$).

In the superficial endometrium the peak at seven hours was 2.1 times higher with the drug combination than with ALA alone ($p<0.01$). The fluorescence at this time was 4.8 times higher than in the myometrium with ALA alone ($p<0.001$); but was 28.9 times higher with the ALA / CP94 combination ($p<0.001$).

In the deep endometrial glands the peak seen with the combination was 1.2 times higher than with ALA alone ($p<0.05$). The fluorescence measurement in the deep glands was 1.9 times higher using ALA alone compared with the myometrium ($p<0.001$); but it was 9 times higher in the deep glands using the combination compared with the myometrium ($p<0.001$).

8.2.7: Results II: Comparison of buffered and unbuffered solutions.

No adverse reactions were observed when administering any of the drugs.

Comparisons of buffered and non-buffered data were made using the buffered data obtained in the first experiment (section 8.2.6). We were unable to use more animals because of restrictions set by the Home Office on the number of animals used. Full results are outlined in appendix 3.

8.2.7.1 *Tissue fluorescence quantification.*

Figure 8.2.12 shows how the fluorescence in the superficial endometrium varied with time between giving the ALA/CP94 combination in either saline or buffer.

Figure 8.2.13 shows how the fluorescence in the deep endometrial glands varied with time between giving the ALA/CP94 combination in either saline or buffer.

Figure 8.2.14 shows how the fluorescence in the deepest endometrial glands varied with time between giving the ALA/CP94 combination in either saline or buffer.

Figure 8.2.15 shows how the fluorescence in the myometrium varied with time between giving the ALA/CP94 combination in either saline or buffer.

All results are tabulated in appendix 3.

The median fluorescence in the superficial endometrium with ALA alone has a peak at six hours with saline (85 a.u.) and seven hours with buffer (48 a.u.). It is 1.8 times higher without buffer ($p < 0.001$). In the deep glands the median peak fluorescence is reached at seven hours with buffer (18.5 a.u.) and six hours with saline (20 a.u.) ($p > 0.05$). Finally in the myometrium the buffered ALA peaks at six hours (20.1 a.u.) and also at six hours with saline (3.5 a.u.) ($p < 0.05$).

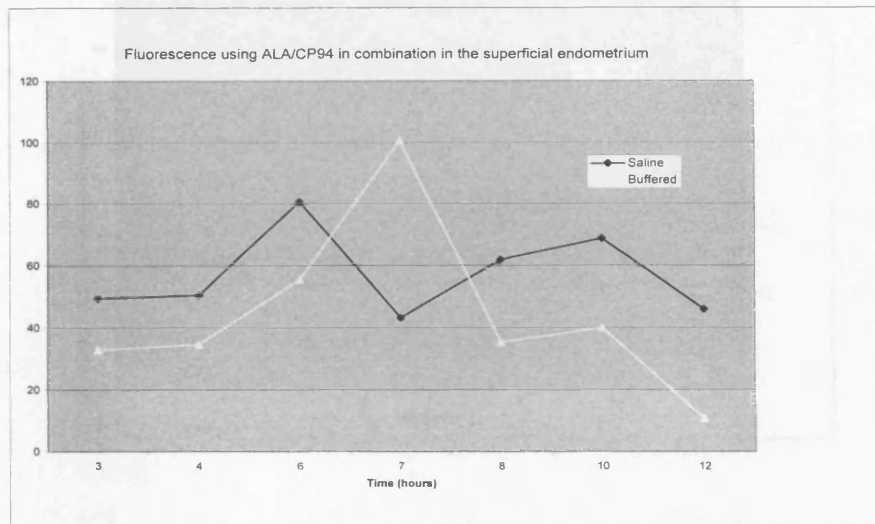


Figure 8.2.12: Fluorescence in the superficial endometrium using the ALA/CP94 combination.

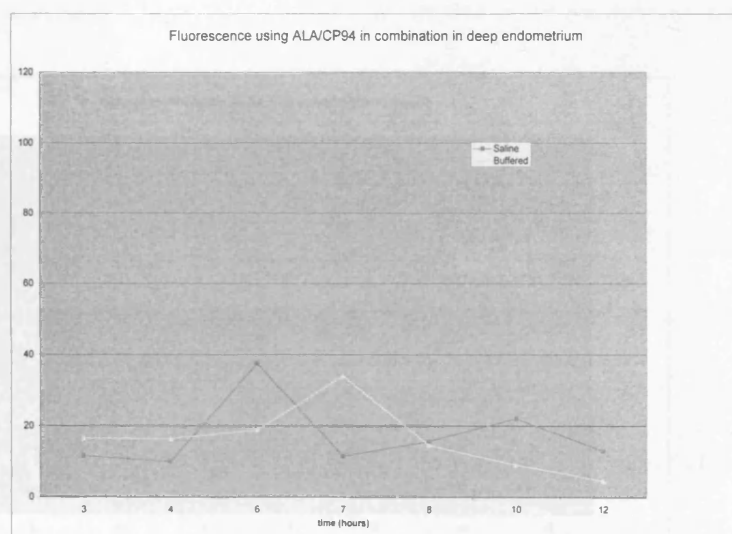
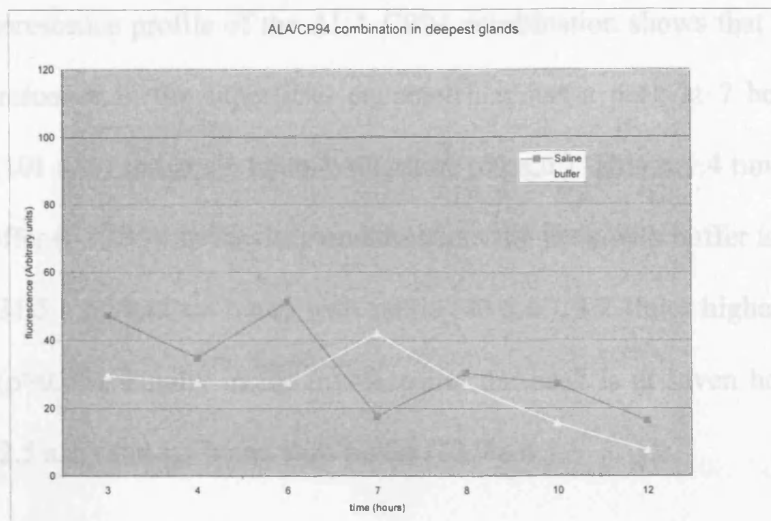


Figure 8.2.13: Fluorescence in the deep endometrium using the ALA/CP94 combination.



8.2.8. Discussion.

The fluorescence microscopy studies of the rabbit uterus show that PPIX accumulates preferentially in the endometrium, with low levels in the myometrium. This is consistent with other workers findings in the rat, rabbit and human (Yang et al 1993, Fehr et al 1995, Garmon 1995). Low levels in the myometrium will minimise PDT effects here and therefore help to maintain physiological integrity, reducing risks of lower transmission and uterine perforation.

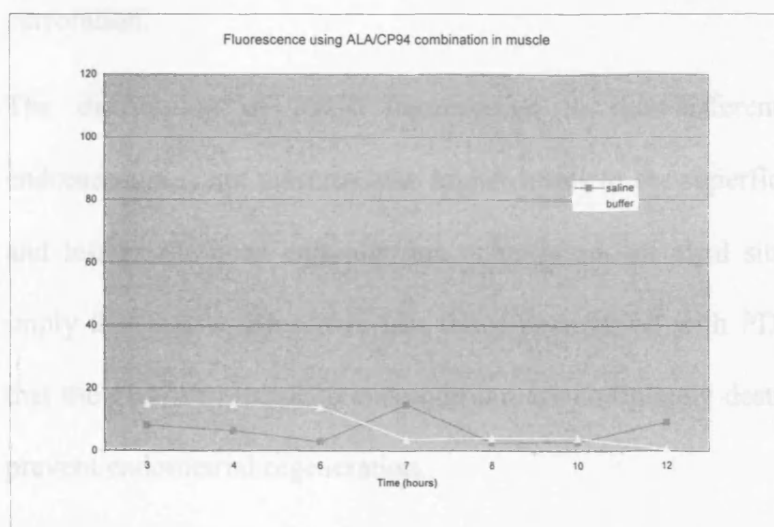


Figure 8.2.15: Fluorescence in the muscle the ALA/CP94 combination.

The fluorescence profile of the ALA-CP94 combination shows that the level of fluorescence in the superficial endometrium has a peak at 7 hours with buffer (101 a.u.) and at six hours with saline (69 a.u.). This is 1.4 times higher with buffer ($p>0.05$). In the deep endometrium the peak with buffer is at seven hours (31.5 a.u.) and six hours with saline (40 a.u.), 1.2 times higher without buffer ($p>0.05$). Finally in the myometrium the peak is at seven hours with saline (2.5 a.u.) and six hours with buffer (13.9 a.u.).

8.2.8 Discussion.

The fluorescence microscopy studies of the rabbit uterus show that PPIX accumulates preferentially in the endometrium, with low levels in the myometrium. This is consistent with other workers findings in the rat, rabbit and human (Yang et al 1993; Fehr et al 1996; Gannon 1995). Low levels in the myometrium will minimise PDT effects here and therefore help to maintain myometrial integrity, reducing risks of laser transmission and uterine perforation.

The distribution of PPIX fluorescence in the different levels of the endometrium is not uniform with higher levels in the superficial endometrium and less in the deep endometrium. This is not an ideal situation as it may imply that this will result in less tissue destruction with PDT. It is essential that the glands in the deep endometrium are completely destroyed in order to prevent endometrial regeneration.

There were significantly higher fluorescence levels in all layers of the endometrium with the ALA / CP94 combination compared with ALA alone. This suggests that there will be greater PDT damage with the combination.

It was interesting to note that the levels were lower in the myometrium using the combination than with the ALA alone. This may be incidental but may be due to less ALA being available because of higher levels absorbed by the endometrium. The results of Curnow et al (1998) using ALA and CP94 given intravenously (i.v.) showed higher levels in the colon with the combination solution.

The highest levels of endometrial fluorescence measured with the ALA / CP94 combination was after 7 hours, the peak absorption using ALA alone was also 7 hours, this is somewhat at odds with other workers (Wyss et al 1994; Steiner et al 1995; Fehr et al 1996). Wyss et al (1994) obtained their results by performing a similar experiment to us. He did however only administer drug at 3, 6, 9 and 12 hours prior to removing the uterine sample. It is possible, therefore that they overlooked the peak. Certainly from our results the peak would have been overlooked if our time points had been greater than two hours apart.

The addition of CP94, therefore, improves both endometrial selectivity of the ALA as well as increasing ALA concentrations in the endometrium. Our data using buffered solutions was at odds with other workers. We therefore had to investigate whether pH would have any effect on the fluorescence.

The Home Office restricts the use of animals and we were therefore limited to using the initial data comparing it to data obtained in a different set of experiments at a different time. This is far from ideal, but after discussion with statisticians at the University and Home Office vets it was deemed acceptable.

Statistical analysis of the data showed significant peaks at different times with different solutions. Comparative statistics have then been used to analyse these data from the peaks. Thus comparison of, for example, the six hour data using ALA and CPC94 in saline were compared with data at seven hours using ALA and CP94 in buffer.

The non-buffered solutions used in these experiments have a pH of 1.5 to 2. This is clearly far from physiological. The assumption is that tissues will absorb a more physiological solution better. The other assumption is that a very acid solution will cause tissue destruction purely because of the pH. In most previous studies of ALA pharmacokinetics or PDT, there is no mention of pH, or that the ALA is buffered with (commercially available) phosphate buffered saline solution PBS (Wyss et al 1992; Yang et al 1993; Fehr et al 1996; Wyss et al 1996). As we have shown from our work the quantities of phosphate required to buffer 200mg / ml ALA are huge, and there is no commercially available PBS that even remotely approaches the concentration of buffer required.

At a pH above 5.0, the ALA solution tends to break down forming a brownish polymeric product. The pH in the lower female genital tract can be quite acidic ranging from pH 3.5 to 4 depending on the menstrual cycle. A very acidic solution may cause a local reaction by the endometrium releasing prostaglandins and causing pain, thus a more physiological solution would be more acceptable.

We hypothesised that using solutions buffered to a more physiological pH would improve the absorption of the photosensitisers. Clearly from our data this is not borne out. The optimum parameters, therefore, appear to be to use

ALA and CP94 in combination dissolved in either saline or a buffered solution, and to perform PDT at 7 hours. It is more acceptable to use the buffered solution because of better patient acceptability.

8.3 Experiment 3: Pharmacokinetic studies of topical ALA and CP94 in the rabbit uterus using different transport mediums.

8.3.1 Introduction.

Traditionally ALA is given in a solution dissolved in saline. Several potential problems arise from this. First, the resultant solution is extremely acidic. However as shown in the last chapter, this appears to be of little relevance to the results obtained. Second the solution is a non-viscous liquid, and therefore has the potential to spill through either the ends of the fallopian tubes (in women) or from the end of the uterine horns in the rabbit model. In our experiments this was reduced by gently tying both the proximal and distal ends of the uterine horns with a silk suture.

When ALA-PDT is used in dermatological cases, the ALA is mixed in a highly viscous cream - Orobace. This allows the drug to be in contact with the skin for long periods of time; at the end of treatment it is easily wiped away.

We wanted to test whether there was a difference in the uptake of ALA by the endometrium when the ALA was mixed with two different substances, namely Orobace and KY jelly. KY jelly is a water-based lubricating gel. We compared these with the (buffered) saline solution.

8.3.2 Animal model.

Three rabbits were used for the Orobace study, four for the KY jelly study. As indicated earlier, because of ethical and licence restrictions the buffer saline results were used from the previous study.

8.3.3 Chemicals.

ALA and CP94 powder were supplied as described above. For this first experiment, the ALA was mixed in a weight to weight manner with the Orobace, the mixture was equivalent to 400 mg per gramme (approximately 400 mg/ml). A second mixture was made up in a similar way using ALA in combination with CP94, to a concentration of approximately 400mg per ml and 100mg per ml respectively. The mixtures did not require buffering as the salts ALA and CP94 powders were not in solution, and therefore there was no dissociation of the salt, and therefore no pH can be recorded.

In the experiment using KY jelly, the ALA or ALA / CP94 powder was mixed with KY jelly resulting in a solution of 400 mg/ml ALA and 100 mg/ml CP94, buffer was added as described above so that the resultant solution was pH 4-4.5.

The mixtures were made up immediately prior to administration. Because of the viscosity of the mixtures, no needles were used, merely the syringe. Due to the viscosity of Orobace, it was heated in a water bath prior to mixing at 38⁰C. The drug was injected through the ends of the uterine horns, the vaginal ends having been closed with a silk tie. After injection the ends were tied with silk ties as before. The total volume given to each uterine horn was 0.5 ml; (200 mg of ALA or a combination of 200 mg of ALA with 50 mg of CP94). Separate syringes were used for the different drug combinations.

8.3.4 Procedures.

Animals were given a general anaesthetic and the operation protocols were followed as described earlier. This facilitated an easier administration. The

animals were recovered and then killed at various times after the dosage (2 - 5 hours). Each uterine horn was excised. The horn was then opened longitudinally, the mixture gently removed, and then the uterine horn was snap frozen in liquid nitrogen. Ten-micrometer thick cryosections were then prepared, with adjacent haematoxylin and eosin (H and E) staining, as described in chapter 7.

The frozen sections were analysed by fluorescence microscopy as described earlier.

8.3.5 Fluorescence Studies.

Phase contrast microscopy with a slow-scan cooled charged-coupled device (CCD) camera was used to image and quantify fluorescence on the frozen sections as previously described. Measurements were made from the superficial endometrial glands (adjacent to the lumen), the deep glands (adjacent to the junction with the myometrium) and from the myometrium of each section. Multiple sections were analysed from each horn.

8.3.6 Statistical analysis.

We discussed the data with our local statistician, and because of the small numbers we have used descriptive statistics only in this section.

8.3.7 Results.

No adverse effects were observed during or after administration of the drugs.

8.3.7.1 Procedural results.

Only three animals were used in the Orobase study and were killed at 2, 3 and 4 hours. Mixing the ALA even with the warmed Orobase proved extremely difficult. Administering the resultant mixture into the lumen of the uterine

horns was next to impossible because of the viscosity. Once the animals were killed it also proved very difficult to remove the Orobase from the delicate uterine horns. In view of this and the fact that no other similar substance was available within the experimental time scale it was decided to abandon any further experiments with orobase.

The KY jelly mixture also proved somewhat difficult to administer. When administered immediately after mixing, the KY jelly retained its viscosity. Yet in a sample made up to see how it reacted over time, there was complete breakdown of the KY jelly to its water-based constituents within ten minutes. KY jelly made without buffering degraded almost immediately. Again after discussion with our pharmacological advisor and the Home Office vet it was decided to terminate the experiment after only using four animals.

8.3.7.2 *Tissue fluorescence quantification.*

This was carried out as previously described.

Figures 8.3.1, 8.3.2 and 8.3.3 shows how the fluorescence induced by ALA and the ALA / CP94 combination in the superficial endometrium, deep glands and myometrium respectively varied with both time and whether orobase or KY jelly were used.

Background autofluorescence has been subtracted from all measurements.

Because of the constraints of numbers and therefore very limited results, we can only make observations. Certainly no conclusions can be made about the peak fluorescence in any level of tissues. We can merely compare each level at different times.

In the superficial endometrium using ALA alone there was no statistical difference between the two preparations in the fluorescence at 2 or 3 hours.

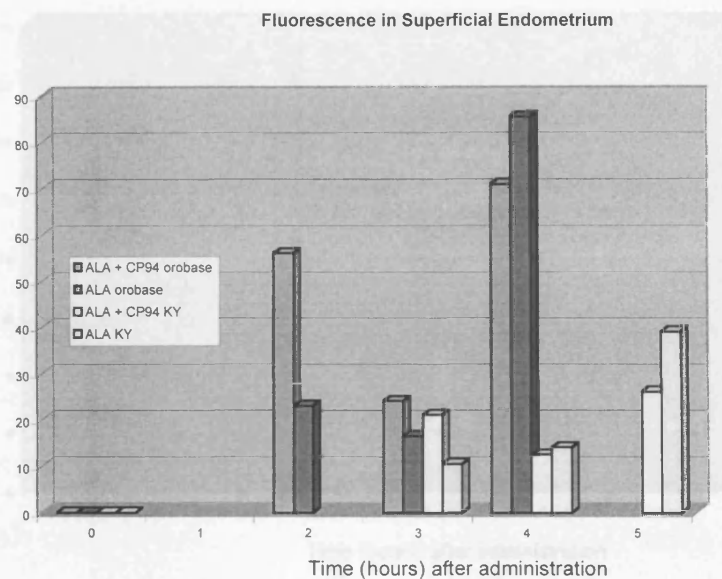


Figure 8.3.1: Variation in fluorescence in the superficial endometrium using ALA and ALA/ CP94 combination with orobase and KY jelly as solvents.

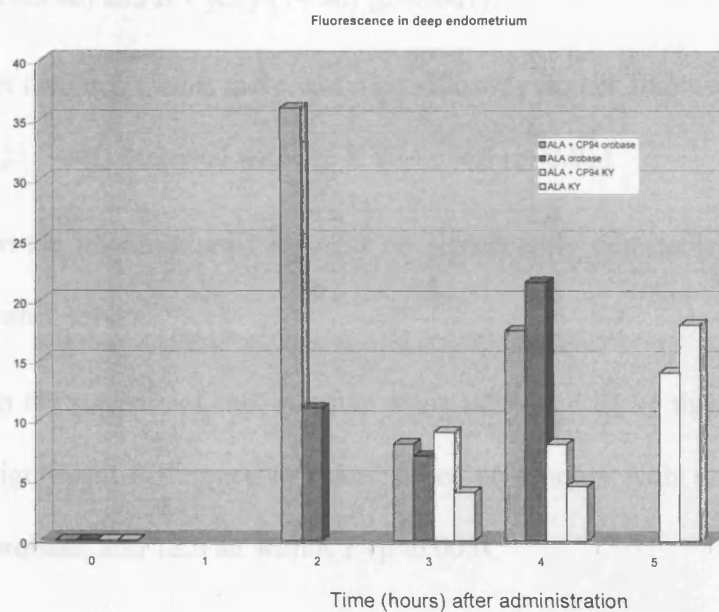


Figure 8.3.2: Variation in fluorescence in the deep endometrium using ALA and ALA/ CP94 combination with orobase and KY jelly as solvents.

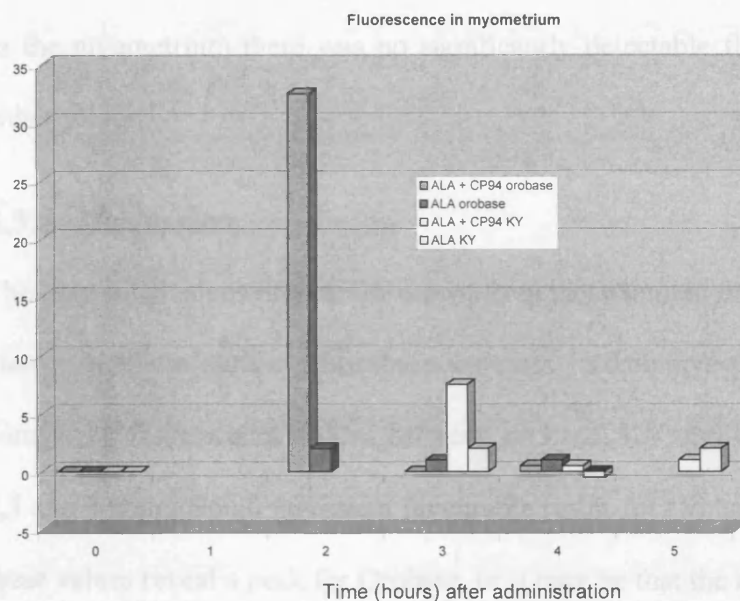


Figure 8.3.3: Variation in fluorescence in the myometrium using ALA and ALA/ CP94 combination with orobase and KY jelly as solvents.

At 4 hours there was a significant difference between orobase (85.5 arbitrary units au) and KY jelly (14 au) ($p < 0.001$).

In the deep glands there was a significantly higher fluorescence using orobase (21.5 au) compared with the KY (4.5 au) ($p < 0.01$).

In the myometrium there was no significantly detectable fluorescence using either solvent.

In the superficial endometrium using ALA and CP94 there was a statistically significant difference in fluorescence at 4 hours with values of 71 au with orobase, and 12.5 au with KY ($p < 0.005$).

In the deep glands there was a significantly higher fluorescence at 4 hours using orobase (17.5 au) compared with the KY (8 au) ($p < 0.01$).

In the myometrium there was no significantly detectable fluorescence using either solvent.

8.3.8 Discussion.

The only conclusions that can be drawn from these limited data is that a highly viscous medium similar to orobase warrants further investigation. Certainly comparing fluorescence values between orobase, KY and buffered saline at 2,3 and 4 hours would indicate a favourable result for Orobase. It may be that these values reveal a peak for Orobase, or it may be that the eventual peak at 7 hours would have been considerably higher than the saline. However, a peak of over 80 a.u. is of the same order as the maximal peak with saline. The advantage of a 4 hour peak is in the time delay between administration and treatment that will be discussed in the final chapter.

CHAPTER 9: PHOTODYNAMIC THERAPY OF THE RABBIT UTERUS USING TOPICAL ALA ALONE AND IN COMBINATION WITH CP94.

9.1 Introduction.

The previous chapters have established that by administering the hydroxypyridinone iron chelator, CP94, it is possible to substantially increase the PPIX fluorescence produced in the endometrium. Also that administering the drug in buffered solution made little difference. This chapter will investigate whether this increased PPIX fluorescence, can be utilised to produce enhanced PDT effects in the uterus.

9.2 Chemicals.

The ALA solutions and the ALA/CP94 solutions were prepared as detailed in chapter 7.

9.3 Animal model.

Normal female New Zealand White rabbits (3 - 4 kg) were used throughout as outlined in chapter 7. There were 22 animals used in this experiment (Table 9.1)

9.4 PDT studies.

The drugs were administered to the uterine horns in the same way as the fluorescence studies in chapter 7. In the saline PDT study, irradiation occurred at the peak fluorescence of six hours after administration of the

drugs. In the buffer PDT study, irradiation occurred at the peak fluorescence of seven hours after administration of the drugs.

Rabbit number	Description	Saline or buffer	Sacrifice (day)
1	Laser only	-	3
2	Laser only	-	3
3	Laser only	-	10
4	Laser only	-	28
5	Drug only	Buffer	3
6	Drug only	Buffer	10
7	Drug only	Buffer	28
8	Drug only	Saline	3
9	Drug only	Saline	10
10	Drug only	Saline	28
11	Control	-	3
12	PDT	Saline	3
13	PDT	Saline	3
14	PDT	Buffer	3
15	PDT	Buffer	3
16	PDT	Saline	10
17	PDT	Saline	10
18	PDT	Buffer	10
19	PDT	Buffer	10
20	PDT	Saline	28
21	PDT	Saline	28
22	PDT	Buffer	28
23	PDT	Buffer	28

Table 9.1: Animals used in these experiments

To irradiate the endometrial cavity, a second laparotomy was performed, again, under general anaesthetic. Two 4cm long diffuser fibres, coupled to a

beam splitter were attached to a 628 nm. diode laser, which was used throughout the studies (figure 9.1). The laser fibre was passed through the ends of the uterine horns. The light dose of 100 J/cm fibre (output was 100mW/cm fibre) was delivered to each uterine horn (figure 9.2). The uterine horns were kept moistened with saline throughout the experiment to prevent drying. At the end of treatment the abdomen was closed in two layers with silk. The animals were then recovered, and killed at three days, and the uterine horns removed, fixed in 10% formalin, and sent for histological examination. Ten micrometer samples were taken and stained with H & E. These specimens were then examined histologically. Twelve animals were used for active PDT in these experiments. In addition two laser only, two drug only and two blank controls were used.

The method of evaluation of the PDT effect was determined as described previously. We wished to have a simple method of testing destruction in a reproducible manner.

An initial general description was made of each histological slide, based on the macroscopic view.

Histological measurements were made using a phase contrast light microscope at a magnification of x16 and x32 using a 0.1mm graticule fitted to the lens piece of the microscope. The thicknesses of the endometrium, including the maximal and minimal thickness of the endometrial folds were measured, and a ratio obtained (figure 9.3). The stromal and the myometrial thickness were measured. The number of glands in the superficial and deep endometrium was noted. For each specimen four separate measurements were made. All the

measurements from horns treated in a similar manner were averaged and a score given of zero, one, two or three.

The number of endometrial glands per square millimetre (mm²) was specifically measured. For each animal, slides were made up from two distinct region of each uterine horn. Five slides were made from each of these regions. Four measurements were made from each slide, and these were averaged. There were, therefore, ten measurements for each uterine horn from each animal. Table 9.2 shows the results from each animal.

9.5 Results.

9.5.1 Macroscopic examination at day three, ten and twenty eight.

Examination of the abdominal organs when the control animals uterine horns were harvested at day three, day ten and day twenty eight showed no damage to adjacent organs. The control uterine horns appeared normal, at all stages. In all the treated animals, the abdominal organs showed no damage, but there were occasional adhesions formed between uterine horns and adjacent structures. In the horns at day three, the uteri were oedematous and erythematous. In many animals the uteri were adherent to adjacent tissues with fine adhesions. There was no serosal damage seen, and all uteri were intact. Examination at later dates showed little or no oedema and the presence of occasional adhesions. At all stages the horns remained intact.

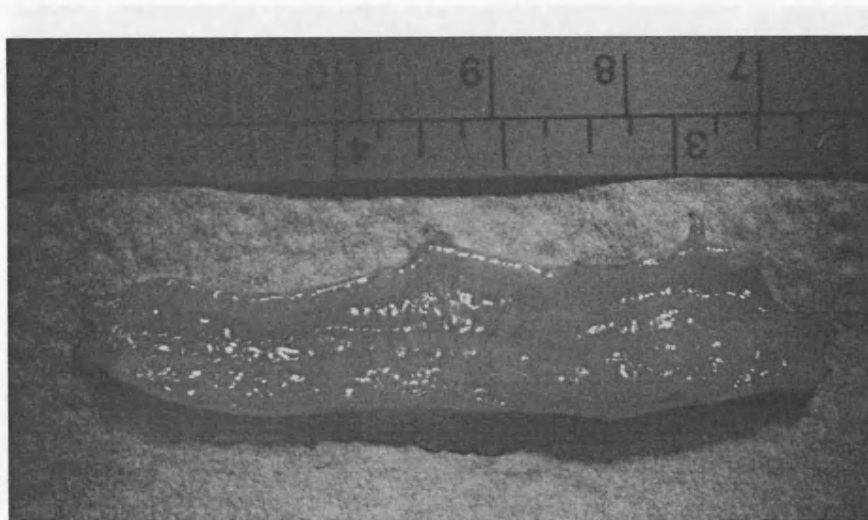


Figure 9.1: Macroscopic damage to the uterine lining at day 3, showing loss of folds after PDT using ALA alone.

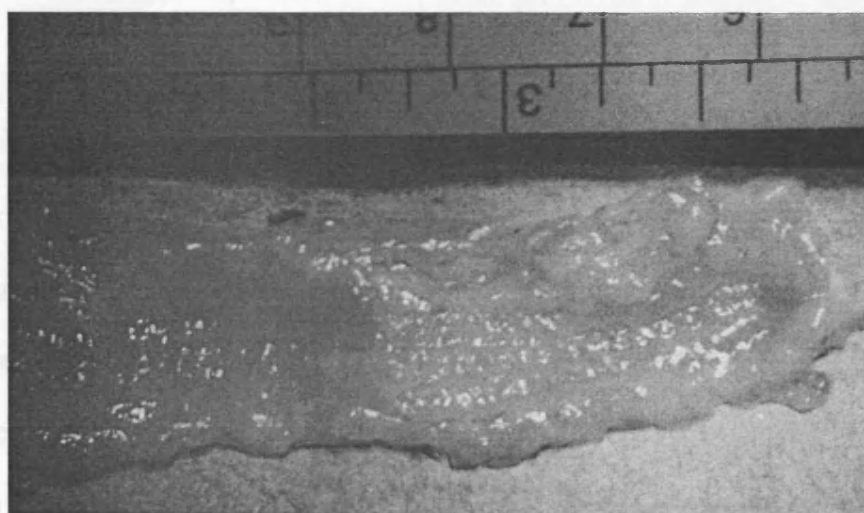


Figure 9.2: Macroscopic damage to the endometrium at day 3, showing loss of folds after PDT using ALA / CP94 combination.

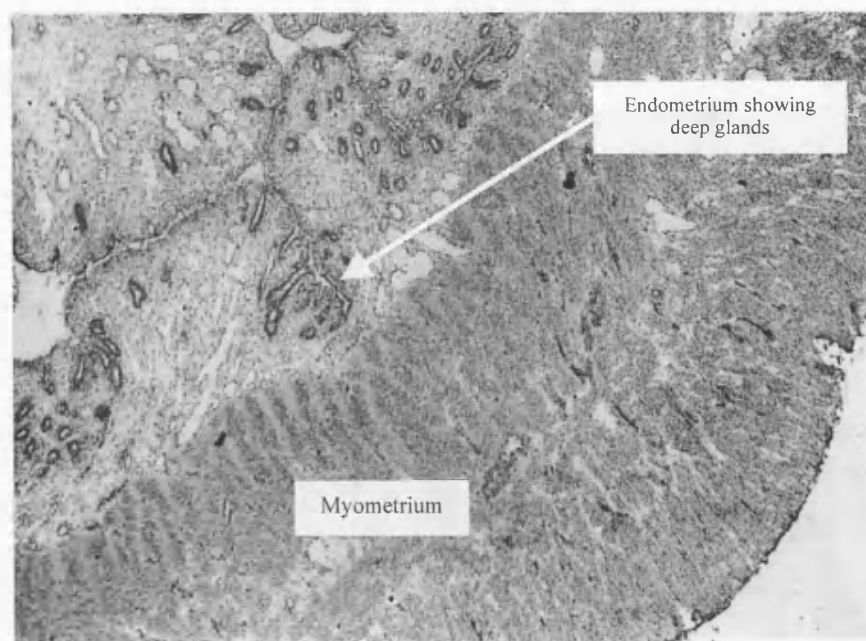


Figure 9.3: Normal rabbit uterine histology.

9.5.1.1 *Control animals.*

Figure 9.3 shows the normal histology of a mature rabbit uterus. The laser only histology and the drug only controls at all time points had similar histology to this. The normal endometrium has folds, with a dense array of glands, both in the superficial, as well as in the deeper endometrium. Deep to the endometrium is the stroma. This consists of a loose extracellular matrix, with fibroblasts and blood vessels of varying size. Deep to the stroma is the myometrium. This layer consists of an inner circular and an outer layer of longitudinal muscle.

Figure 9.5: Histology of day three after ALA/CP94-induced PDT.

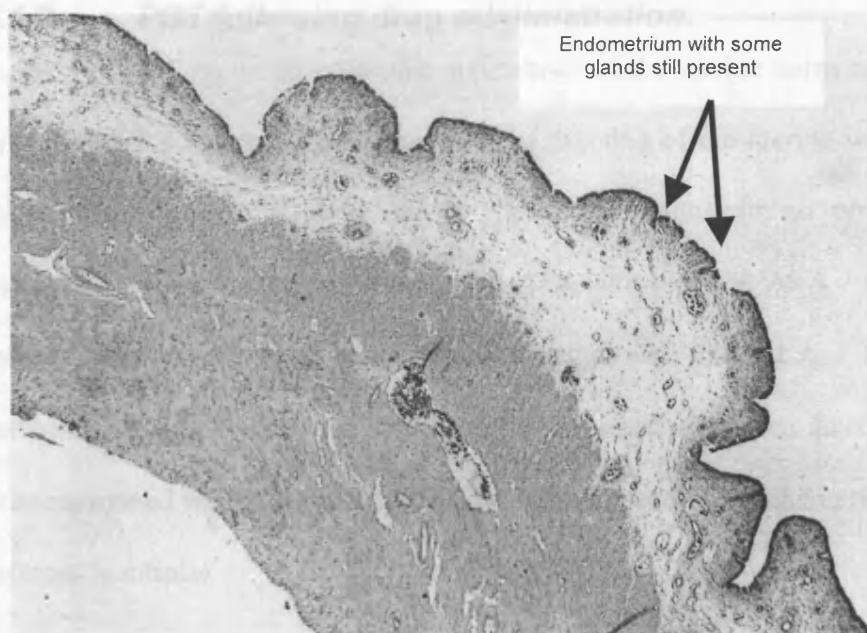


Figure 9.4: Histology at day three after ALA-induced PDT

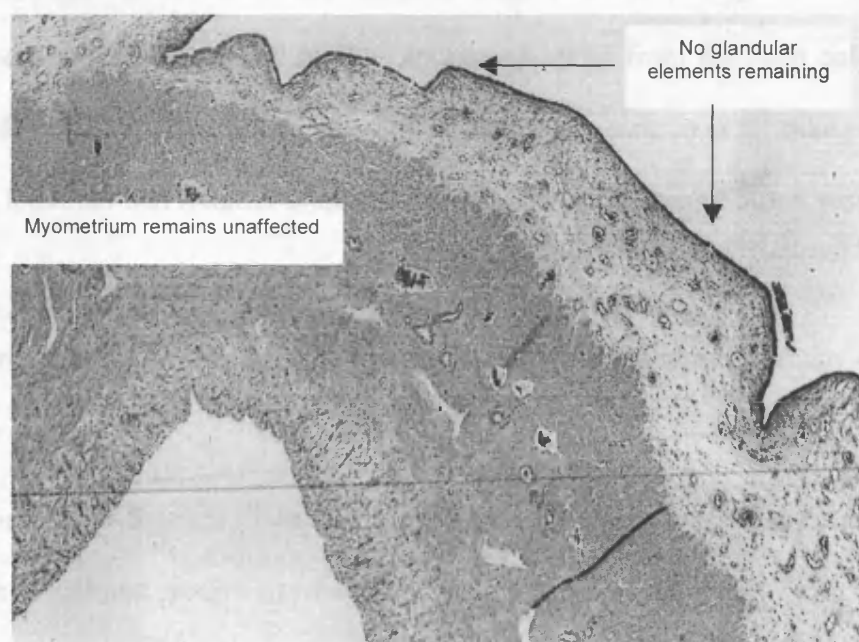


Figure 9.5: Histology at day three after ALA/CP94-induced PDT

9.5.1.2 *PDT following drug administration.*

The effect of PDT on the macroscopic appearance of the uterine horns can be seen in figure 9.4 and 9.5. There was obvious thinning of the uterine wall in both specimen groups at three days. There were similarly no obvious differences between the horns treated with ALA alone or the ALA / CP94 combination at ten days. The specimens treated with the ALA / CP94 combination at four weeks was similar to the specimens from ten days. The specimens treated with ALA alone were similar in appearance to the untreated specimens (controls).

9.5.2 Microscopic examination.

9.5.2.1 *Control animals.*

Figure 9.3 shows a representative H and E histology slide of a normal rabbit uterus. The luminal surface of the untreated uterus shows numerous thick endometrial folds. These are covered with a columnar epithelium with numerous glandular openings. The specimens taken from the light only and the drug only specimens are all identical to this appearance at all time points. The loose stromal tissue is thick and contains occasional small blood vessels.

9.5.2.2 *PDT following drug administration.*

There were no differences between the buffer and saline groups at any of the time points.

Figure 9.6 to figure 9.11 show representative histological sections from each of the treatment groups: day three, ALA alone and in combination with CP94; day ten, ALA alone and in combination with CP94; day twenty eight, ALA alone and in combination with CP94.

9.5.2.2.1 Day three

There were a few differences between the ALA alone and ALA / CP94 combination treatment groups at three days. The morphology of the endometrium was completely changed following PDT in all specimens, compared to the normal. There was extensive haemorrhage in the endometrium, with epithelial and blood vessel damage. The myometrium showed minimal haemorrhage. There were occasional inflammatory cells present in the stroma.

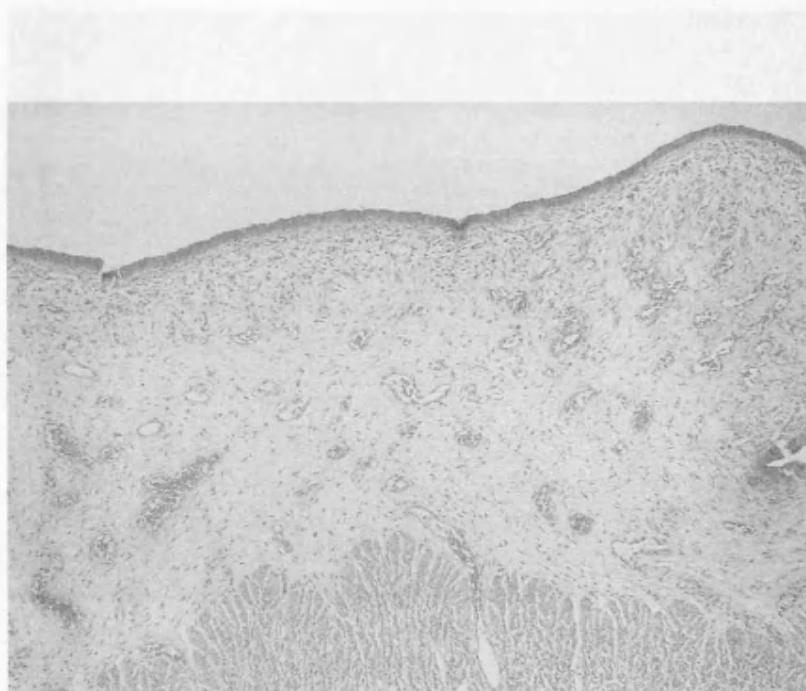
In the ALA treated uterine horns, there was marked endometrial damage, with extensive loss of folds, thinning of the endometrium, and a marked reduction in the number of superficial endometrial glands. Although in some specimens glandular destruction was seen throughout the endometrium, in the majority of specimens these deeper glands appeared almost unaffected.

The epithelium was of a cuboidal type, in the majority of the superficial endometrium. There was occasional loss of epithelial cover. Although in some ALA treated specimens glandular destruction was seen throughout the endometrium, in the majority of specimens these deeper glands appeared almost unaffected. The columnar epithelium persisted in the majority of the residual folds of the endometrium.

In those horns treated with CP94 in combination with ALA, there were obvious morphological differences between those treated with ALA alone. There was endometrial thinning with quite extensive flattening. Surface epithelium where present was cuboidal, but there was extensive loss of epithelial cover. Generally there were no endometrial glands seen.



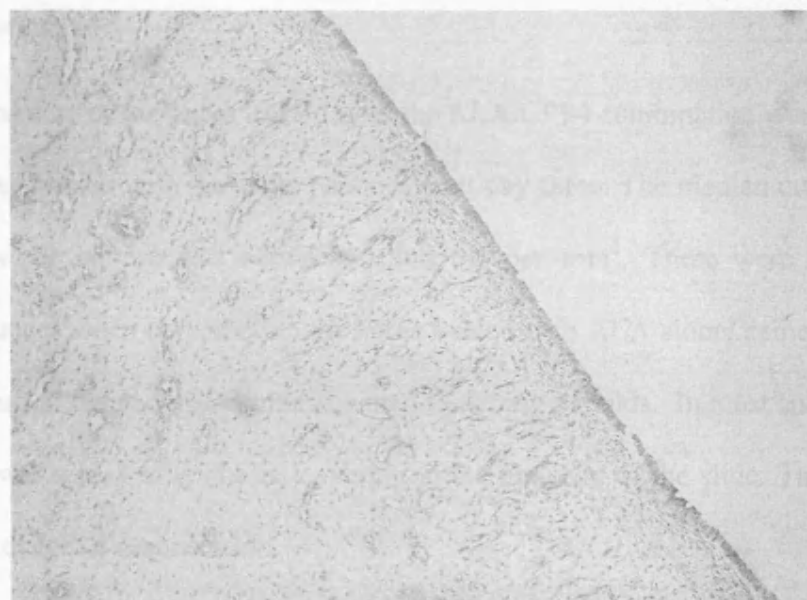
Figure 9.6 (a) and (b): Representative histology at day three after ALA-induced PDT



9.5.2.2 Day five



Figure 9.7 (a) and (b): Representative histology at day three after the ALA/CP94 combination-induced PDT



9.5.2.2.2 Day ten

The endometrial morphology was markedly different than normal. There was no obvious difference between the histology using the different drug combinations comparing saline and buffered solutions.

A large degree of regeneration had occurred in the uteri from the horns treated with ALA-induced PDT alone, with a median number of glands of between 0 and 1 per mm², but also with an increase in the depth of glandular penetration. There was also a generalised thickening of the stroma. There was moderate endometrial damage, with extensive loss of folds, thinning of the endometrium, and a marked reduction in the number of superficial endometrial glands, as seen at day three. However, the majority of the epithelium was of columnar type. There was generally a reduction in the number of endometrial glands, especially in superficial endometrial, but again, in the majority of specimens these deeper glands appeared unaffected, or had regenerated.

Morphology of the horns treated with the ALA/CP94 combination was similar to those treated with the same parameters at day three. The median number of glands per animal was between 0 and 0.5 per mm². There were obvious differences when compared to the horns treated with ALA alone, namely gross thinning of the endometrium associated flattening of folds. In most specimens there was a loss of epithelial covering in the majority of the slide. There was no evidence of regeneration.

In no specimen was there evidence of myometrial damage.

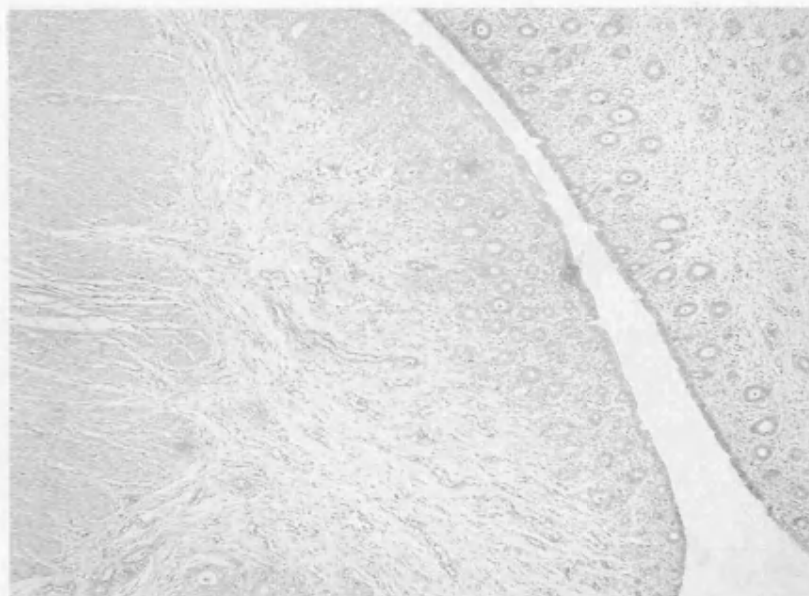
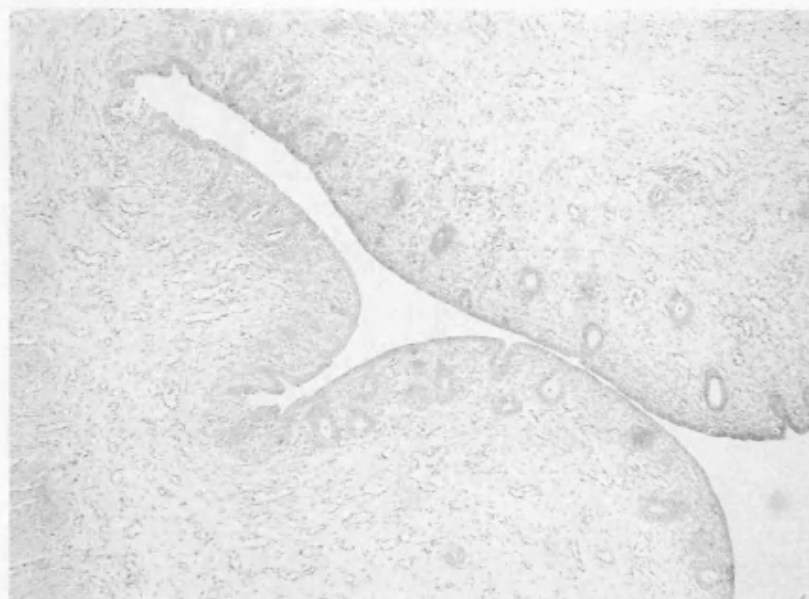


Figure 9.8 (a) and (b): Representative histology at day ten after the ALA-induced PDT. There is glandular regrowth.



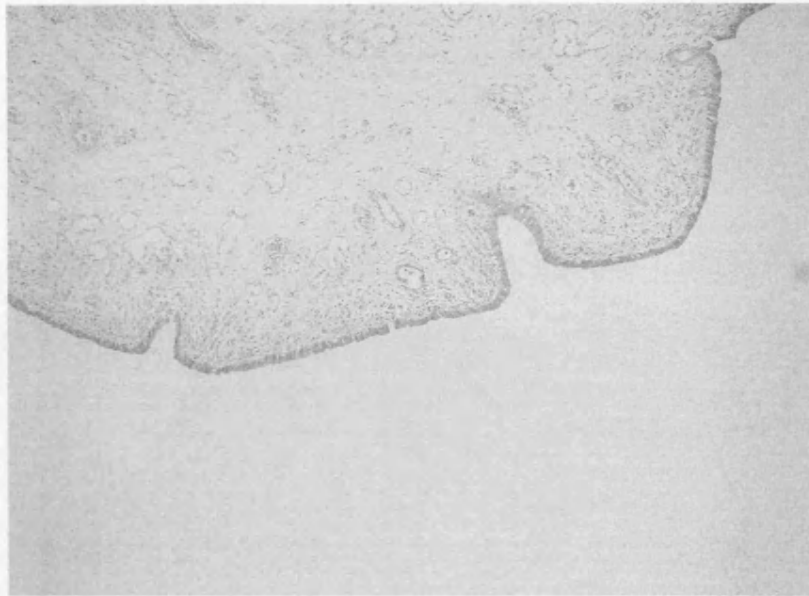
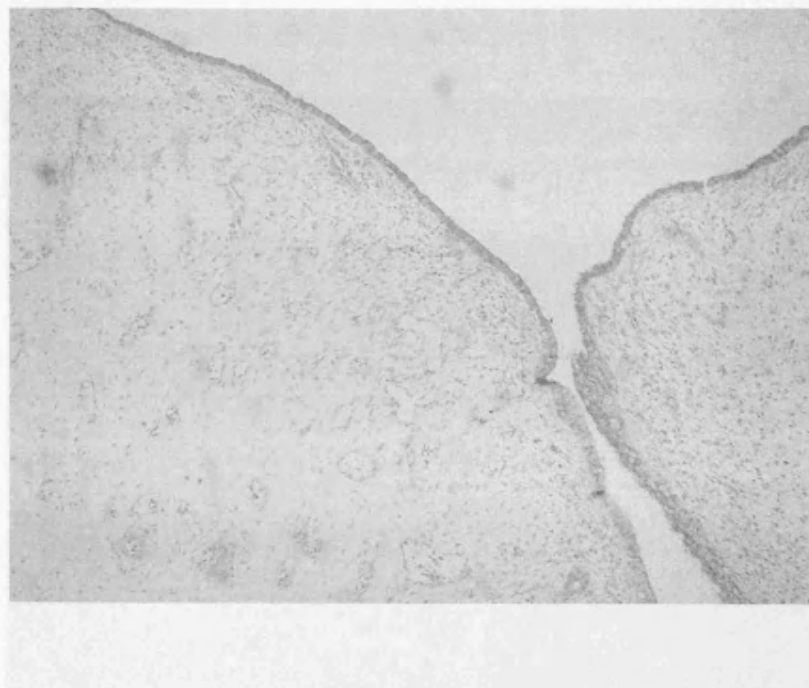


Figure 9.9 (a) and (b): Representative histology at day ten after the ALA/CP94 combination-induced PDT. There is no glandular regrowth



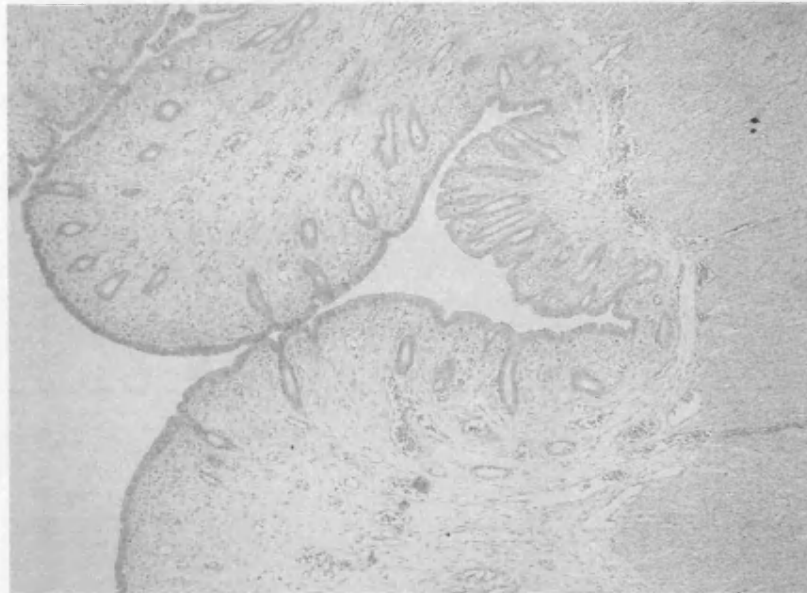


Figure 9.10 (a) and (b): Representative histology at day twenty eight after ALA-induced PDT, showing considerable regrowth of glandular elements



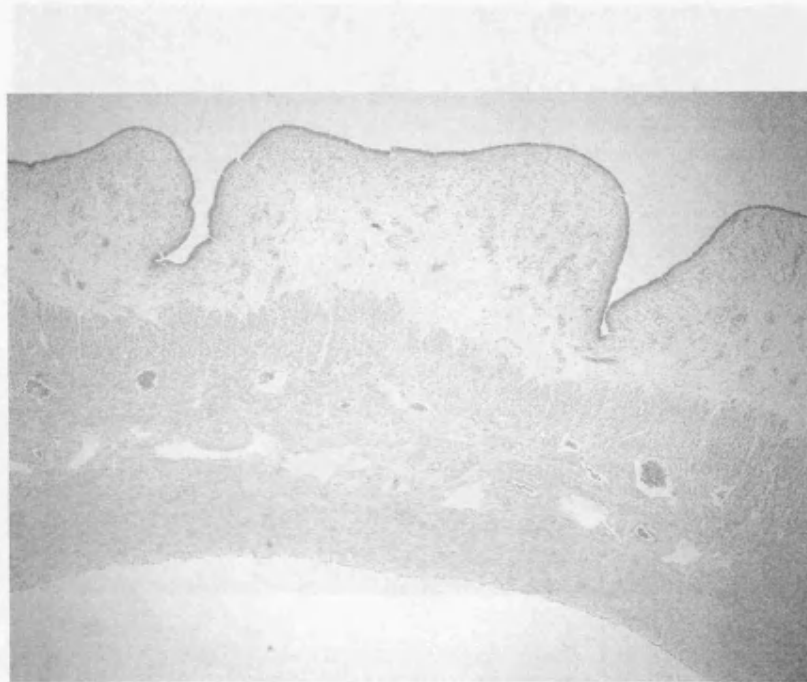


Figure 9.11 (a) and (b): Representative histology at day twenty eight after the ALA/CP94 combination-induced PDT showing a denuded endometrium with no regrowth of glandular elements

Endometrial effects four weeks after PDT

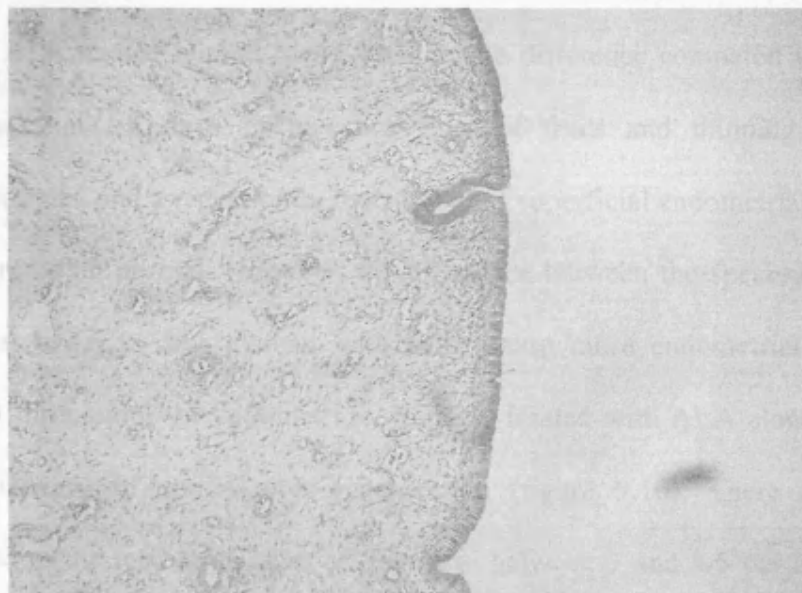


Figure 9.12: Representative histology at day twenty eight after the ALA/CP94 combination-induced PDT showing a denuded endometrium with no regrowth of glandular elements



Figure 9.11 (c): Representative histology at day twenty eight after the ALA/CP94 combination-induced PDT, showing some regrowth of glandular elements.

9.5.2.2.3 Endometrial effects four weeks after PDT.

In the ALA treated uterine horns, there was a difference compared with the specimens at ten days. There was loss of folds and thinning in the endometrium, and a reduction in the number of superficial endometrial glands compared with normal. However, the difference between the specimens and those at ten days was evident with there being more endometrial glands present throughout the endometrium in those treated with ALA alone, there was evidence of considerable regeneration (figure 9.10). There was an increase in the median number of glands to between 3 and 4.5 per mm². In some of the specimens there was complete regeneration of tissue.

Animal number	Description	Endometrium			Myometrium
		Overall score	Overall score ALA alone	Overall score ALA/CP94	Overall
1	Laser only 3	+			No damage
2	Laser only 3	0			No damage
3	Laser only 10	0			No damage
4	Laser only 28	0			No damage
5	DO B 3		0	0	No damage
6	DO B 10		0	0	No damage
7	DO B 28		0	0	No damage
8	DO S 3		0	0	No damage
9	DO S 10		0	0	No damage
10	DO S 28		0	0	No damage
11	CONTROL		0	0	No damage
12	PDT S3		+++	+++	No damage
13	PDT S3		++	+++	No damage
14	PDT B3		+++	+++	No damage
15	PDT B3		+++	+++	No damage
16	PDT S10		+	++	No damage
17	PDT S10		0	+++	No damage
18	PDT B10		++	+++	No damage
19	PDT B10		+	++	No damage
20	PDT S28		0	++	No damage
21	PDT S28		0	+++	No damage
22	PDT B28		0	++	No damage
23	PDT B28		+	+++	No damage

(number = sacrifice day; DO = drug only; S = saline; B = buffer)

Table 9.2: results of the experiment (with reference to table 7.1).

In contrast, the endometrium of the horns treated with the drug combination remained thin, with little regeneration. However, as can be seen in the example shown in figure 9.11(c), there were some areas of regeneration. The

median number of glands was between 0.5 and 1.5 per mm². There were two exceptions. Again, there was no myometrial damage seen in any specimen.

9.6 Discussion.

In order to prevent regeneration, endometrial destruction with photodynamic therapy must extend within the endometrium to include the deep glandular tissue.

PDT after ALA was administered alone, shows extensive endometrial damage. However, there were a greater number of both superficial and deeper endometrial glands remaining when compared with the specimens after PDT with the drug combination. The current limited and unsustained clinical effects of PDT to the human endometrium after topical administration of ALA (used alone) may be explained by these findings.

It is known that endometrial regeneration occurs within a few days, from residual glands deep within the endometrium, whether after normal menstruation, curettage or after endometrial destruction, by means explained previously (Ferenczy 1976a; Ferenczy 1976b). In order to prevent regeneration glandular elements throughout the thickness of the endometrium must be destroyed. Evidence from our histology of the ALA/CP 94 combination, shows complete endometrial destruction.

Topical application of ALA in the uterus reduces systemic levels and therefore may eliminate systemic side effects, notably skin photosensitivity (Roberts et al 1989; Dougherty et al 1990). By giving two 0.5 ml doses of the ALA solution, the animal will have a total dose of approximately 400 mg. This is equivalent to approximately 100 mg / kg. This compares favourably with the

i.v. administration used by Yang of up to 400 mg / kg (Yang et al 1996). Wyss instilled ALA into the rabbit uterine horns, using 1.2 ml of up to 400mg / ml. This is equivalent to a dose of 240 mg / kg (Wyss et al 1994). The doses used topically in rats by Wyss were up to 400 mg / kg (Wyss et al 1994). Yang also used ALA topically in rats using 0.1 ml per horn at doses of up to 160 mg / ml. This is equivalent to doses of up to 640 mg / kg (Yang et al 1993).

The rabbit endometrium is very thin compared to that of the human. Previous animal experiments with ALA PDT have shown excellent results using laser energy at the optimum wavelength for PPIX of 635 nm, but when the technique has been taken into the clinical setting, the results have thus far been disappointing. This may be due to insufficient PPIX concentration in the deepest endometrial glands resulting in incomplete ablation of these glands from which regeneration occurs. This may, therefore, explain the poor clinical results obtained with this technique. By combining CP94 with the ALA a higher local concentration of PPIX in the endometrium can be obtained than has been previously possible. We have demonstrated an improved photodynamic effect.

A larger difference was shown in the PDT effects between ALA alone and the ALA/CP94 combination in the rabbit model, because the laser used was a 628 nm laser rather than a 635 nm laser. Previous work in this department has shown that photodynamic destruction using a laser is 2.5 times greater at 635 nm than at 628 nm ($p < 0.02$) (Curnow 1999).

The rabbit endometrium has numerous deep folds, similar to the fallopian tube in humans, and very different from the relatively smooth human endometrium. The bare diffusing fibre that we used meant that the endometrium would have

remained folded, resulting in a reduction of illumination to the glands within the deep folds. Better results may have been obtained if we had utilised a laser diffuser fibre within a distendable balloon. Despite this, PDT after instillation of the drug combination showed good endometrial destruction and the long-term results are encouraging.

CHAPTER 10: LIGHT DISTRIBUTION IN THE HUMAN ENDOMETRIAL CAVITY.

10.1 Introduction.

In the previous chapters, it has been shown that there is complete and long-standing damage to the endometrium of the rabbit after photodynamic therapy using topical ALA combined with CP94. In these experiments a single 4 cm laser diffusing fibre was used. Within the rabbit uterine horn this system offers adequate illumination of the entire horn (essentially a hollow cylinder). The human uterus, however, has the shape of an inverted flattened pear. The endometrial cavity as the shape of an inverted triangle with a narrow cavity transversely, being described as a potential cavity (figure 10.1). Placing a single diffusing fibre in the endometrial cavity, without distension, would therefore fail to adequately illuminate all parts of the endometrium. For PDT to be offered as a successful treatment of menorrhagia, not only should endometrial destruction be full thickness, but also it must be total.

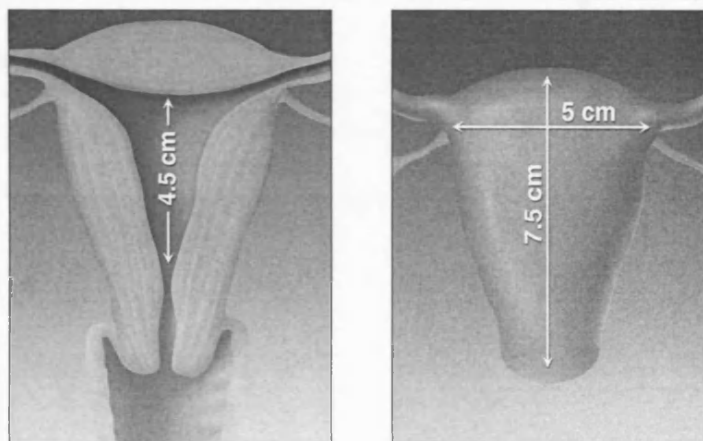


Figure 10.1: transverse section of human uterus.

The light delivery device is, therefore, of crucial importance. Tromberg used mathematical models to show that sufficient light propagation through the uterine cavity is possible with three cylindrical fibres (Tromberg et al 1996). Due to the fact that some form of cavity distension is necessary to allow light to penetrate all areas, we looked at the possibility of developing an intrauterine balloon. The distension pressure within the balloon must not be so great as to prevent adequate oxygenation of the tissue, crucial for PDT to occur, by restricting the blood supply. In order to fit the human endometrial cavity, the balloon must be of a similar shape to the cavity - namely an inverted flattened triangle. It was not known whether this non-uniform shape would in turn lead to problems with uniformity of light distribution. In clinical practice, placement of the balloon and laser fibre in the uterine cavity is likely to be performed as a blind procedure. It is also important that consistent and accurate placement is easily attained.

The ideal light delivery system should be easy to place within the cavity and should allow consistent laser fibre placement within the cavity. If we assume that a balloon device is used, there must be uniform light intensity over the entire surface.

10.2 Materials and Method.

10.2.1 Modified Wilson-Cook Uterine balloon stent.

The first device is our own design (Figure 10.2) in which a cylinder diffuser fibre is placed within a transparent Balloon Uterine Stent (Cook Ob/Gyn, Spencer, Indiana), The Cook balloon is a 60 x 45 mm flattened transparent silastic balloon, designed to be used to tamponade the endometrium to reduce bleeding. We modified the balloon by the addition of different proximal

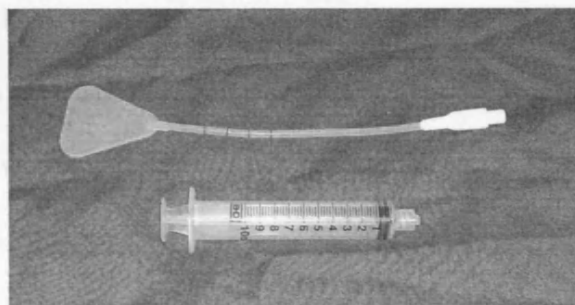


Figure 10.2a Unmodified Wilson-Cook balloon

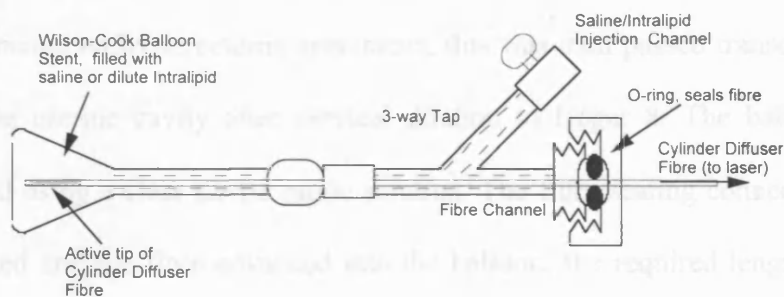


Figure 10.2b Modified Wilson-Cook balloon

applicators. For normal use the Cook balloon has a one-way valve, which for our purposes is removed. We needed to fit a three-way tap to allow the saline distension medium to be instilled. We also fitted a modified foley catheter tip on one arm of the three-way tap. This was needed to insert the laser diffuser fibre. The internal diameters of the channel of the tap used were less than 1 mm. The increased size was a very simple modification. By using a small amount of glycine as a lubricant we were able to insert the laser fibre with ease. The glycine also allowed the system to be water-tight with a minimal amount of saline loss after instillation. The geometry of the cylinder diffuser does not match the uterine cavity, so the primary illumination at the endometrium is non-uniform. Light is backscattered and reflected within the uterine cavity, which should lead to improved uniformity of illumination of the endometrial surface.

The balloon was connected to the three-way tap and the cylinder diffuser fibre was partially inserted into the fibre channel and secured in place with an O-ring, which also prevented leakage of fluid. The deflated balloon was clamped in a Spencer Wells forceps, and wrapped around it. For our experiments on hysterectomy specimens, this was then passed transcervically into the uterine cavity after cervical dilation to Hegar 8. The balloon was inflated using a clear sterile saline solution. The fibre sealing connection was loosened and the fibre advanced into the balloon, the required length having previously been marked.

10.2.2 Medlight balloon.

The Medlight balloon (Medlight SA, Eclubens, Switzerland) has a 5.3 mm diameter, 150 mm long introducer to pass the folded balloon transcervically.

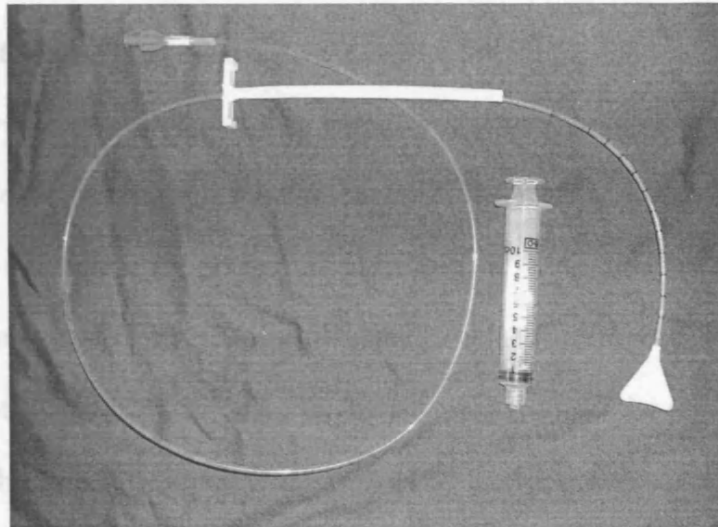


Figure 10.3: Medlight balloon

10.2.4 Methodology.

The balloon is made of a white translucent latex in the shape of a 'typical' uterine cavity (figure 10.3). It is inflated with the recommended 2 ml of saline, and attains dimensions of 25 x 25 x 5 mm. The catheter is 100 mm long. The design uses a high internal reflectance for the balloon material to improve the uniformity of light distribution, based on the same principle as an integrating sphere (i.e multiple internal reflections redistribute the light more uniformly on the tissue surface). In theory, this should give much improved performance over a transparent balloon. The fibre is partially loaded into the device and secured in place by tightening the sealing connection. The deflated balloon is smeared with a lubricating jelly and passed into the introducer, which can be

relatively easily inserted via the cervix without dilation. The introducer is withdrawn and the balloon inflated using a clear sterile saline solution. The fibre sealing connection is loosened and the fibre advanced into the balloon, the required length having previously been marked.

10.2.3 Uterine specimens.

A total of 7 premenopausal and 2 postmenopausal patients scheduled for hysterectomy for benign conditions such as menorrhagia and prolapse, were recruited into the study. Nine intact, fresh uteri were obtained fresh from surgery and placed in saline. The uterine vessels were tied to minimise leakage of blood from the tissue. During the experiment, the uteri were kept moist with saline. The study was approved by the local ethics committee, and all women were counselled and consented for their uterus to be used in the study.

10.2.4 Methodology.

The specimens were gently held by clamps in the laboratory. The cervix was dilated to 6mm and a flexible 5mm hysteroscope with a light source was used to view the cavity. The cavity was checked for uniformity, and pathology (polyps, significant fibroids or suspicious lesions) (figure 10.4). Five 16G cannulae were passed through the myometrium from the outside (figure 10.5). The needle inserts were withdrawn when the needles were seen within the cavity with the hysteroscope. Detection fibres (500µm) were passed through the cannulae under hysteroscopic control.



Figure 10.4: Checking uterine cavity with a hysteroscope.

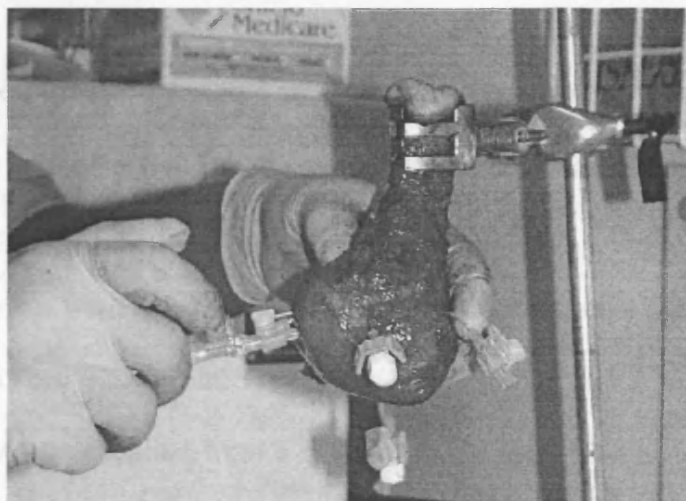


Figure 10.5: Inserting fourth cannula. The placement of the others has already been checked with the hysteroscope.

The positioning of the five cannulae is shown in the diagram (figure 10.6).

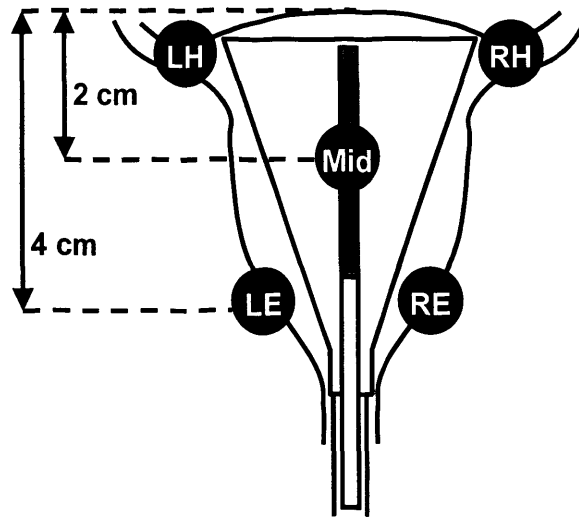


Figure 10.6: Approximate positioning of cannulae and isoprobe detectors within the uterine cavity.

The detection fibres were placed an estimated 1-2mm within the cavity. Once all five had been placed and checked, the hysteroscope was withdrawn. One of the balloon devices or the bare fibre was then inserted into the cavity. The balloon devices were filled with saline. The amount of saline inserted varied, but was between 1.5 to 3 ml for the Medlight balloon and 2 to 8ml for the Cook. Light was supplied from a 630 nm diode laser (Diomed, Cambridge UK). Power output was 100 mW. A 2 cm long, 400 μ m core diameter radially diffusing fibre was positioned inside the devices and used to illuminate the cavity (RD-20 Medlight SA, Eclubens, Switzerland) (figure 10.7).

Optical fibres with spherical, isotropically diffusing tips were used at the locations shown in figure 10.6. Each had an 800 μ m diameter scattering bulb

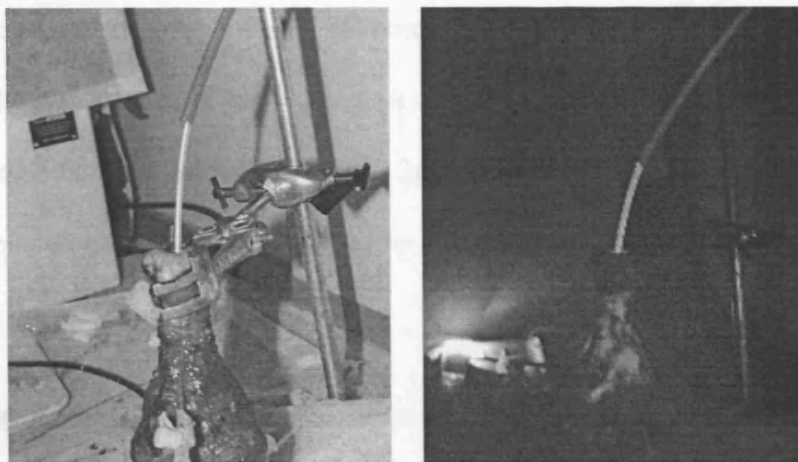


Figure 10.7: Uterine cavity with (a) laser inserted and (b) being illuminated with laser light.

(DP1 Isoprobe, PDT Systems Inc., Santa Barbara, CA). These detectors measure the total power arriving at the bulb from all directions, which is the quantity of interest in PDT (light from any direction will activate the photosensitiser). The isoprobes were connected to a CCD spectrograph (Control Development, South Bend, Indiana) - each individual measurement was a spectrum in the range 625 - 635 nm and the power was estimated as the integral under the laser peak in this wavelength range. This was done for several reasons: the CCD array has high sensitivity and a very wide signal range and it allowed compatibility with other studies (including fluorescence measurements) undertaken with the same equipment. The isoprobes were introduced through the wall of each uterus through the plastic cannulae.

During the balloon introduction, the isoprobe bulbs were retracted within the cannulae, and they were advanced to their locations at the endometrium when

inflation was complete. This protected the fragile bulbs from damage during insertion and inflation of the balloons. The depth of insertion for each isoprobe was ascertained using a hysteroscope before starting the measurements so that approximately 2mm were within the cavity, and flags were placed on the isoprobe fibres to ensure these depths were maintained during the measurements.

10.2.4.1 Data collection.

The raw data were collected using the CCD spectrometer as described. To convert these data to power readings, the background was subtracted from each spectrum. The power was calculated as the integral under the laser peak in the area under this baseline-corrected spectrum between 625 and 635 nm. Integration times were 0.01s, 0.025s and 0.05s, this allowed corrections to be made, to a standard integration time of 0.05s. Therefore, readings taken at 0.01s were multiplied by 5 and readings at 0.025s were doubled. Extraneous light is excluded from the CCD as only light at this particular wavelength is measured. Cross-calibration of the data were required as we were using five isoprobes. This corrected for variations in the light fibre collection properties. This was done by the use of an integrating sphere coupled to the laser (figure 10.8). The individual isoprobes were then placed in the detector and the laser fired at specific power settings. A calibration curve was therefore generated for each isoprobe. With each fibre this was a straight line. The calibration factor was taken as the slope of this curve. The corrected data were then obtained.

For each measurement, when the balloon and isoprobe locations had been confirmed, the laser was activated and the power measurements were taken.

The balloon was then deflated and removed. The fibre placement was checked with the hysteroscope, and the balloon device re-inserted and the measurements repeated to assess reproducibility.

At the end of the experiments the uterus was opened with a longitudinal incision in the lateral wall. The uterine dimensions, macroscopic appearance and presence or otherwise of fibroids were noted.

The uteri were transferred to the histopathology department for fixing in formalin and histological assessment.

10.3 Results.

Three of the specimens had such significant fibroids that the cavities were highly abnormal and they were excluded from the main results, but the results are discussed later. The remaining six uteri weighed 420-680 g. The internal transverse diameters of the cavities were 24 - 56 mm. The internal longitudinal lengths of the cavities from fundus to internal os were 62-104 mm. There were small fibroids in a further two specimens; one with three fibroids of less than 10mm, distorting the cavity, the other with one 11mm fibroid and another 4mm fibroid <50% within the cavity. Apart from fibroids there were no abnormal pathological findings. The endometrium was little disturbed and the gynaecological pathologist was satisfied with her results.

10.3.1 Cook balloon.

Uniformity from the transparent Cook balloon was comparable to the MedLight balloon - implying that scattering from the tissue was sufficient to redistribute the incident light effectively (figure 10.8). In fact more light reaches the uterine horns (40% of maximum, c.f. 20% with MedLight).

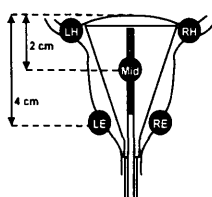
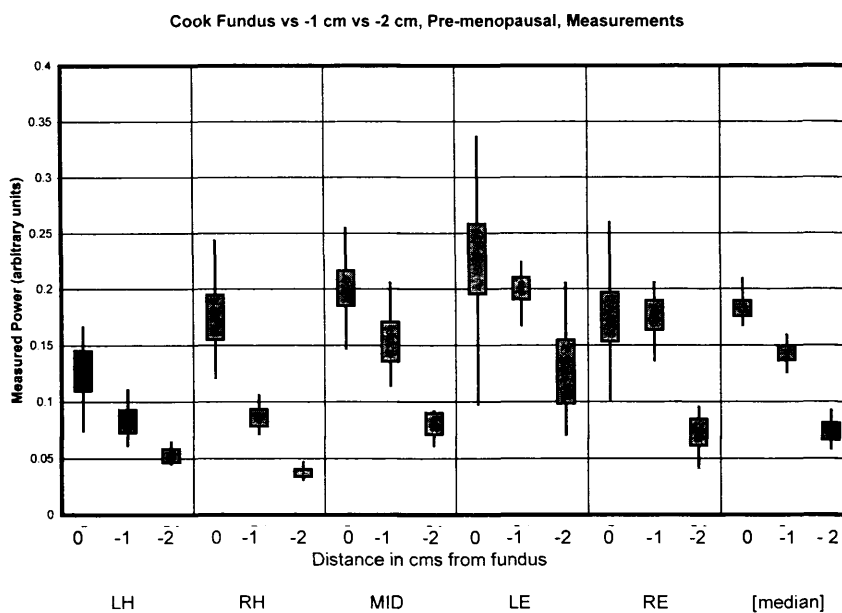


Figure 10.8: Cook balloon data. Laser fibre is placed at fundus and withdrawn one and two cms

Throughput is also higher. The larger balloon fits the cavity better, although when fully inflated the pressure may reduce blood flow and hence oxygen concentration and the PDT effect.

10.3.3 Medlight balloon.

Uniformity is good (figure 10.9), but less light reaches the horns of the uterus (20% of maximum). This may be because the balloon is too small to fill the whole cavity, reducing the transmission efficiency to the fundus of the uterus. The device is very easy to use. Throughput is lower than the transparent

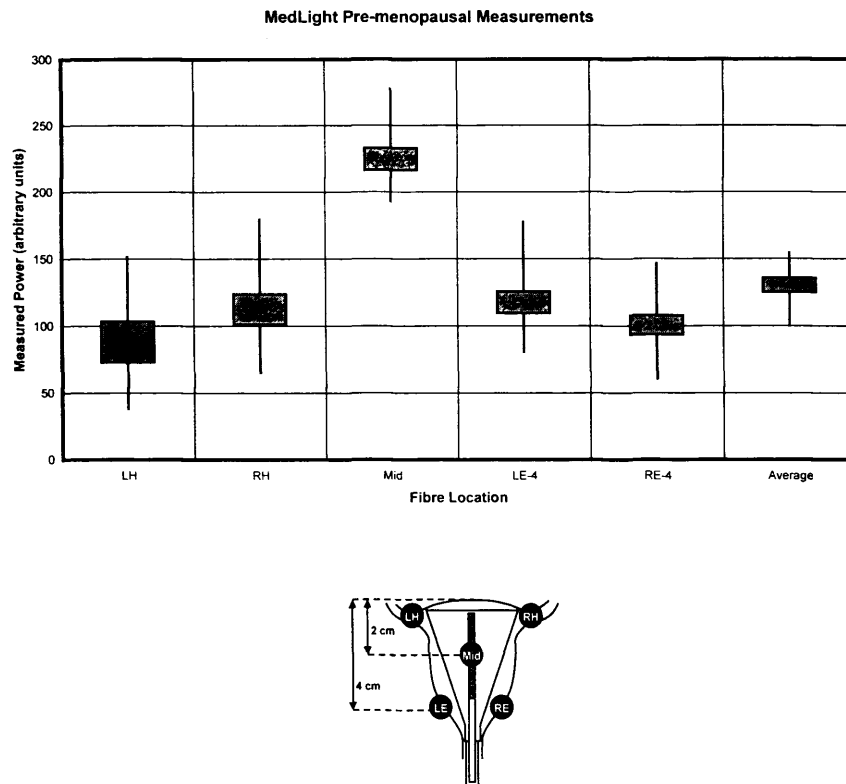


Figure 10.9: Medlight balloon data. Balloon is placed at fundus.

balloon because the high internal reflectance reduces transmission to the tissue.

One postmenopausal uterus which was the smallest in these experiments, and had internal diameters less than the Cook balloon, functioned with no problems with the Medlight balloon, but split as soon as we attempted to inflate the Cook balloon.

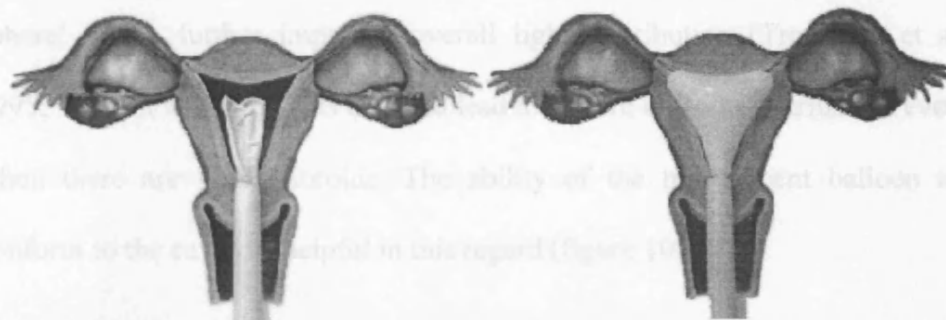


Figure 10.10: Deflated and inflated Medlight balloon.

10.4 Discussion.

Complete and uniform illumination of the endometrial cavity is crucial for PDT to deliver complete endometrial ablation with no subsequent regeneration. Improvement in light distribution within the bladder has been shown with fluid distension (Marynissen et al 1989). Fehr et al showed that by using three or four fibres in the cavity, that no distension is required as long as the cavity is completely normal; even then, a small volume of fluid can improve uniformity of illumination by up to four times. Limitations of filling the cavity with fluid are several. Spillage of fluid either through the fallopian tubes or through the cervix would result in non-standard distension, unless an automated, possibly pressure-driven, distension device was used. Distension with high volumes can lead to pain. Unless integrated, a second insufflating device would need to be employed. In practical terms a balloon, with a closed system would be ideal. This could easily be pressure-monitored. By utilising a small balloon device, the uterine cavity can be distended and because of the

natural reflectiveness of the endometrium can be used as an ‘integrating sphere’ which further improves overall light distribution (Tromberg et al 1995; Wyss et al 1995). This can also lead to a more uniform distribution even when there are small fibroids. The ability of the translucent balloon to conform to the cavity is helpful in this regard (figure 10.10).

The Cook balloon was too large and too rigid to be a successful intrauterine balloon for PDT. The medlight balloon, despite being smaller than the uterine cavity had a broadly similar light distribution characteristics, within each uterus. When we removed the Medlight balloon and reinserted and continued with the experiment, the results again were similar. This was certainly not the case with the Cook balloon, which had highly varied results at each position, which altered considerably with re-insertion. Several modifications will be required to the Cook balloon if this device is to progress to clinical use. It needs an introducer. It needs a stiffer catheter with a larger internal diameter but the balloon itself should be thinner and more flexible.

The Medlight Balloon device has been used clinically elsewhere. Although in theory its characteristics are inferior to the Cook balloon, it is likely to be used for the first clinical applications because of its ease-of-use.

The presence of a submucous fibroid had a significant effect on the results. Our aim was to use a balloon to mould to the cavity, to use the inherent reflective capabilities of the endometrium and the translucent nature of the balloon to cause a fairly uniform glow within the uterus. Our hope was that this would result in illumination of all parts of the cavity.

By using a single fibre, there are three potential problems. First, there is a potential problem of the diffuser fibre being off-centre and thereby part of the endometrium furthest from the fibre will have less illumination. Second, maximal illumination will be concentrated around the centre of the cavity, in order to adequately illuminate all areas of the cavity, a higher power would have to be used, this in turn may lead to thermal effects. Third, in order to get a maximal PDT effect with a single fibre the treatment time may be prohibitively long.

CHAPTER 11: CONCLUDING REMARKS.

11.1 Objectives.

The minimally invasive treatment of menorrhagia has been a goal since ancient times. In the Bible abnormal menstrual blood loss required atonement by a priest;

‘And if a woman has a discharge of blood for many days on the eighth day she should take two turtle doves or two young pigeons, and bring them to the priest, to the door of the tent of meeting; and the priest shall offer one for a sin offering and the other for a burnt offering; and the priest shall make atonement for her before the Lord for her unclean discharge.’

Leviticus Chapter 15; v: 25-30.

The aim of this study was to cause the selective destruction of the endometrium, without causing myometrial damage, by photodynamic therapy using topical ALA applied transcervically. We looked specifically at increasing the photodynamic effect of ALA using a novel iron-chelating agent, CP94. There is a need to improve on the currently available techniques for the treatment of menorrhagia.

We will outline currently available minimal access techniques, and then discuss the possible future place of PDT.

11.2 Other minimal access surgical treatments.

The endometrium has a remarkable capacity to regenerate, and any surgical procedure to treat excessive uterine bleeding, without a hysterectomy must ensure that there is adequate destruction of the basal glands of the endometrium. There are many techniques available. Some are well-established and well researched. Others are still at the research or evaluation stage. A summary of the ‘successes’ of these devices is outlined in table 11.1.

MAS technique	Success (%) (oligomenorrhoea and amenorrhoea unless stated otherwise)	Patient satisfaction
Uterine curettage	60 (but short-term)	
ELA (endometrial laser ablation)	85-90	60-70
TCRE (Transcervical resection of the endometrium)	80-85	60-70
HTA (Hydrothermal ablation)	40	60-75
Thermal balloon	83	80-90
ELITT (endometrial laser interstitial thermal therapy)	60-70 (amenorrhoea)	80
MEA (Microwave endometrial ablation)	40 (amenorrhoea)	80
Cryoablation	28 (amenorrhoea)	88
Diathermy	40 (amenorrhoea)	60

Table 11.1: Success and satisfaction rates with the newer MAS techniques – see text.

The rates of patient satisfaction are reasonably high, despite low amenorrhoea rates, as amenorrhoea is not necessarily the desired outcome. Women often

want a reduction in MBL, without resorting to hysterectomy. There is a correlation between oligomenorrhoea or amenorrhoea rates and satisfaction, for all modalities (Garry 2000). Nevertheless, there are drawbacks to most methods, particularly in long term efficacy.

11.2.1 Uterine curettage.

Uterine curettage was discussed in chapter 3. There is well documented evidence that there is an associated reduction in MBL in the first few months after the procedure of up to 60% (Mikuta 1970), however the effects are short-lived (Haynes et al 1977). It is a relatively simple procedure to carry out, but complications include uterine perforation and intrauterine adhesions or synechiae. Due to the overuse of this procedure the Royal College of Obstetricians and Gynecologists (RCOG) issued guidelines in 1994 (Table 3.2) (RCOG 1994).

11.2.2 Endometrial laser ablation (ELA), rollerball ablation and transcervical resection of the endometrium (TCRE).

ELA was first reported by Goldrath in the late 1970s (Goldrath et al 1981). The first electrosurgical resection was described in 1983 (DeCherney and Polan 1983). TCRE was made popular in the UK by Magos (Magos et al 1989). A simpler technique than loop resection is rollerball ablation (Vancaille 1989). Since then a great expansion in the use of the technique has occurred, driven by both gynaecologists wishing to perform these operations and demand from patients. Numerous publications of uncontrolled series followed (Garry et al 1991; Magos 1991). These are, therefore, not 'new' techniques. A majority of the new methods employed for endometrial destruction have been compared with these 'gold standards'.

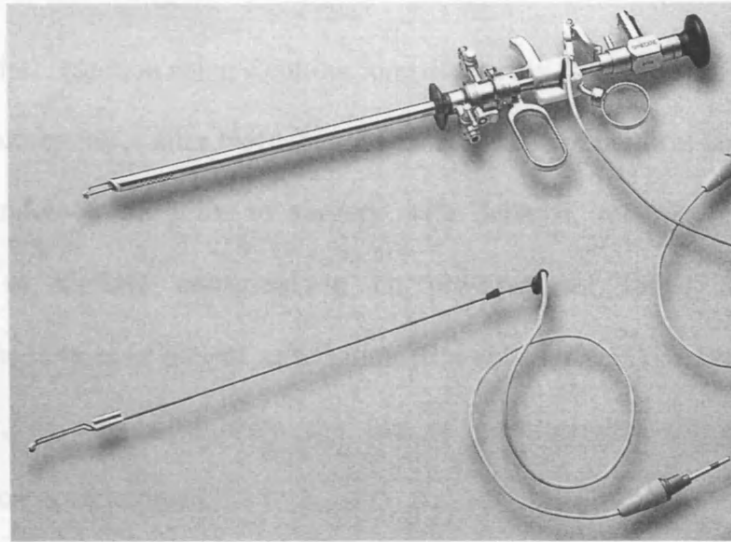


Figure 11.1: Gynaecological resectoscope.

The standards that these techniques have set are high. Most authors use “success” to mean patient satisfaction. Goldrath described his ten-year data in 1989 (Goldrath 1989), reporting a 93% “success rate” from laser ablation. Garry et al reported a 97% success rate, although 8% required a second procedure (Garry et al 1991); the rate of amenorrhoea was 60%. Erian reported on 2342 women with a 93% success rate and 56% amenorrhoea rate (Erian 1994).

Outcomes after rollerball ablation for menorrhagia are similar. Short-term data are available comparing rollerball to hysterectomy. With follow-up of at least 18 months, Mousa showed satisfaction rates of 100% with hysterectomy and 79% with rollerball. He also reported that 100% of the women in the The choice of whether to use laser ablation or endometrial resection, is really the surgeon's, based on his or her skill as well as the availability of equipment.

hysterectomy group would recommend it to a friend and 91% in the rollerball group (Mousa et al 2001).

Endometrial resection using a cutting loop diathermy (figure 11.2) has reports of 80% success rates after more than 2 years. The use of medical pre-treatment of the endometrium prior to surgery with danazol, medroxyprogesterone acetate or GnRHa compared to no pre-treatment failed to improve amenorrhoea rates or patient satisfaction (Rai et al 2000; Trivedi et al 1999). However, in clinical practice the use of pre-treatment for endometrial preparation is widespread.

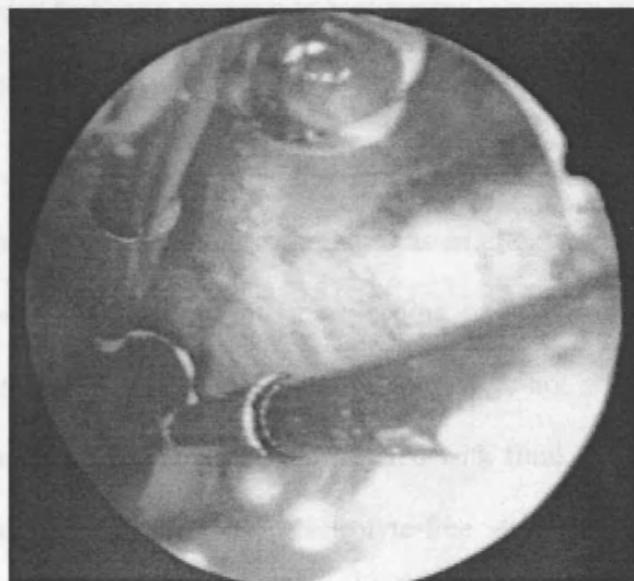


Figure 11.2: Resectoscope showing loop cutting endometrium.

The choice of whether to use laser ablation or endometrial resection, is really the surgeon's, based on his or her skill as well as the availability of equipment.

No randomised-controlled studies have been published. A large study by the Royal College of Obstetricians and Gynaecologists (RCOG) of the minimally invasive surgical techniques, laser, endothermal or endoresection (MISTLETOE study Overton et al 1997) - has shown a 60-70% satisfaction rate in women undergoing either combined techniques, or resection, or laser or rollerball alone. This is coupled with amenorrhoea rates at one year of between 68% (for laser alone) and 85% for the combined resection and rollerball.

A recent study of women randomised into medical management or endometrial resection for menorrhagia showed that women allocated to the surgical management arm were in better general health (Cooper 1999). The study concluded that early recourse to hysteroscopic surgery will 'afford these women better relief of symptoms and improvements in health related quality of life'.

These minimal access techniques were used as an alternative to hysterectomy in women with dysfunctional uterine bleeding or menorrhagia, resistant to medical treatment. These techniques are, however, not without their risks. They require the uterine cavity to be irrigated with fluid. For ELA, saline is generally used. For TCRE, an electrolyte-free solution is used, usually glycine. Fluid overload is potentially fatal, hence careful attention to fluid balance must be paid (figure 11.3).

Hysteroscopic surgery is associated with complications. The most serious, common complication is perforation of the uterus (Lewis 1994). It occurs either whilst dilating the cervix or with the insertion of the hysteroscope. It occurs in approximately 1-2% of cases (Hulka et al 1995; Lewis 1994).

Approximately 50% of all perforations occur during the operator's first three cases. After a perforation it is usual to perform a laparoscopy to assess bleeding, generally nothing more needs to be done. If, however, the perforation has occurred after the resection has commenced, there is a serious risk of extrauterine organ damage, and a laparoscopy or even laparotomy is mandatory.

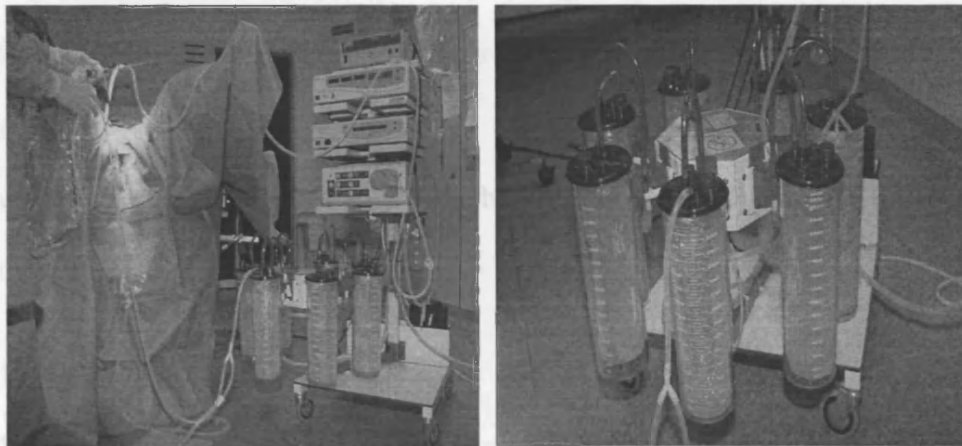


Figure 11.3: Careful fluid balance during a TCRE

Significant fluid overload occurs in approximately 1-3% of cases. Different figures are quoted, and it is in a large way preventable by using a 'running balance'. However, should large vessels be breached deep in the myometrium, there will be rapid fluid absorption. Excess absorption leading to fluid overload may be minimised by giving frusemide intraoperatively. Excess glycine absorption may give rise to life-threatening hyponatraemia, with

symptoms of hypertension, bradycardia, confusion, seizures, coma and death, unless recognised and treated. Death may occur in this acute situation with a sodium of 120 mmol/l (Witz et al 1993). Healthy young women may absorb 1.5 l of glycine with only minor effects. Close monitoring of fluid inflow and outflow should be done during these procedures. Above a deficit of 1000 ml, the procedure should be stopped, and serum sodium should be measured to detect significant hyponatremia, and the woman should be carefully monitored.

Serious haemorrhage after endometrial resection requiring hysterectomy occurs in 1% of cases (Overton et al 1997). The bleeding can be controlled using a foley-type catheter inflated within the uterus. The tamponade is slowly reduced 24 hours later.

11.2.3 Versapoint.

Versapoint (Gynaecare Inc., Menlo Park, California, USA) is a bipolar device where the active and return electrodes are consolidated into a single instrument. The current is symmetrically distributed through the tissue between the two electrodes, the patient is therefore not part of the current pathway. The electrosurgical generator determines the power administered based on the impedance of the tissue. The device channels current into tissue vaporizing it. The devices are 1.6 mm (5 F) in diameter, and fit down the operating channel in a standard hysteroscope. There are different electrodes designed for variable tissue effects, a ball tip for precise vaporization and desiccation, a spring tip for rapid tissue vaporization and desiccation and a twizzle tip for vaporization and needle-like cutting (Figure 11.4).

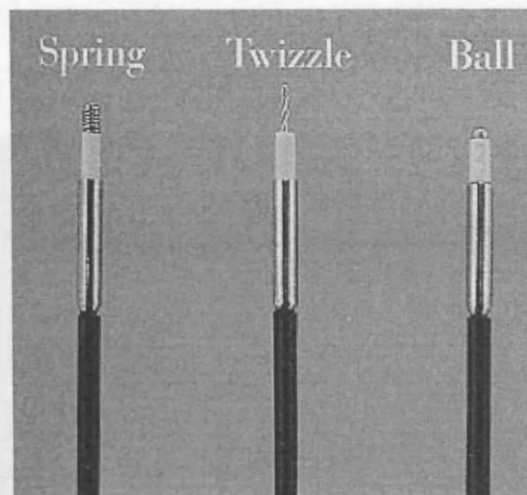


Figure 11.4: Versapoint tips.

Given the small size and focused tissue effects, this system is aimed at outpatient procedures such as hysteroscopic polypectomy, adhesiolysis and vaporization of smaller submucous myomata. It is also available as a resectoscope to be used as for TCRE. It uses saline as it requires a conducting fluid. Saline is a safer medium to use than glycine.

11.2.4 Hydrothermal ablation (HTA).

This technique (BEI Medical Systems, Hackensack, New Jersey) requires a specialised hysteroscopy. Endometrial destruction is achieved by circulating hot saline through the inflow of an 8mm hysteroscope. After the procedure the cavity is smooth with little scar tissue formation long-term, unlike TCRE. This allows the procedure to be repeated (figure 11.5). The system operates at a pressure of 50mmHg. No spillage is seen from the fallopian tubes at this pressure (Richart et al 1999). Results of amenorrhoea at 12 months of 40% are therefore 'single-use' systems. Once activated, the circulating fluid is

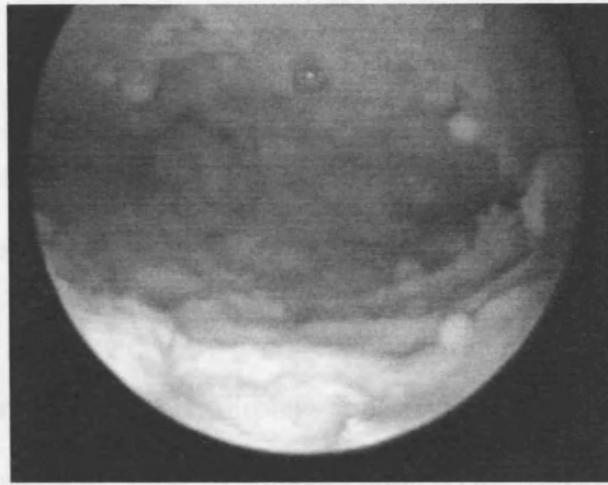


Figure 11.5: Uterine cavity after HTA

11.2.5 'Blind' procedures - non-hysteroscopic surgery.

In recent years there has been a huge expansion of 'blind' transcervical techniques of endometrial destruction. Device manufacturers are driving much of this technology; few have been evaluated thoroughly in randomised trials.

11.2.5.1 Thermal balloon.

There are two thermal balloon devices currently available; Cavatherm and Thermachoice. Essentially they are all very similar, consisting of a strong, flexible and distendable silicone balloon, at the end of an introducing handle. The devices are about 16 cm long and 6-8 mm in diameter. The balloon is passed through the cervix, inflated with fluid to a pressure of 150-200 mmHg, causing it to conform to the uterine cavity. The systems are disposable and are therefore 'single-use' systems. Once activated, the irrigating fluid is

vigorously circulated within a closed system, the temperature of the fluid is maintained at 75 - 85 °C. The treatment cycle is 15 minutes (Friberg et al 1996).

The ThermaChoice uterine balloon therapy system (Gynecare Inc., Menlo Park, California, USA) is one such device. It consists of a 16 cm long, 4.5 mm diameter catheter with a latex balloon at its distal end which houses a heating element.

The controller unit monitors, displays and controls the preset intra-balloon pressure, temperature and treatment time. The device automatically deactivates if the pressure falls below 45 mmHg or rises above 200 mmHg. The cervix is dilated to 5.5 mm. The balloon inserted so as to touch the fundus, and then inflated with 5% dextrose in water until the intrauterine pressure reaches 150-170 mmHg - this typically requires 10-15 ml of solution. The heater is then activated and maintains the intra-balloon temperature at 87°C ($\pm 5^{\circ}\text{C}$). The treatment cycle of 8 minutes results in 4 - 6 mm of tissue destruction (Neuwirth et al 1994).

CavaTherm (Wallsten Medical, Morges, Switzerland) is a similar device (figure 11.6). Thermachoice has been investigated and published data are available on several hundred women (Amso 1998). CavaTherm has had less investigation, but a series of almost 50 women has been reported (Friberg 1998). Patient satisfaction rates of 80 - 90% are reported, but with amenorrhoea rates of only 15%.

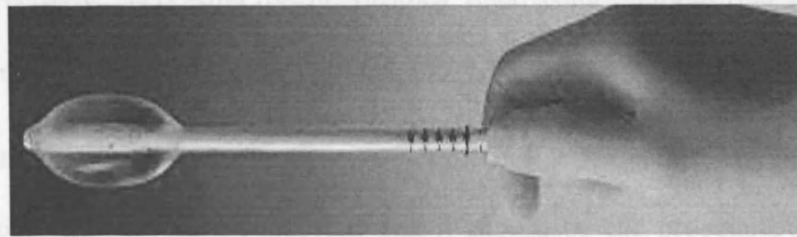


Figure 11.6: Cavatherm balloon

11.2.5.2 Thermal Laser Therapy.

The endometrial laser interstitial thermal therapy (LITT) system (Sharplan Laser Ltd, Weymouth, Dorset) is a form of endometrial laser therapy. One randomised trial has compared the thermal balloon with rollerball ablation in the USA (Meyer et al 1998). The entry criterion was true menorrhagia, based on a pictorial chart; women with fibroids were excluded. No endometrial preparation was used, but all women underwent curettage immediately prior to treatment. Almost half the women in the balloon arm were treated with only local anaesthetic.

Satisfaction rates were equivalent at about 80%. Amenorrhoea rates were 15% with the balloon and 27% with the rollerball, but menstrual scores were significantly less with rollerball than with the balloon (the main outcome measure). Dysmenorrhoea was also significantly reduced with both techniques. Gervaise compared ThermaChoice with endometrial resection (Gervaise et al 1999). There was no difference between the two groups in relation to complications, morbidity or success rates. Success was counted as elimination of menses or a 'significant reduction of flow'. At 24 months success for the balloon was 83% and 81% for the resection group. At 36 months the rates were 83% and 76% respectively (Gervaise et al 1999). Loffer's three year study comparing rollerball and ThermaChoice showed

little difference between the two groups at treating menorrhagia, avoiding hysterectomy, decreasing dysmenorrhoea, decreasing premenstrual symptoms and improving quality of life (Loffer 2001).

Due to the lower complication rate with balloon ablation compared with resection, authors have suggested that it should be the procedure of choice (Loffer 2001; Bongers et al 2000).

11.2.5.2 Thermal Laser Therapy.

The endometrial laser interstitial thermal therapy (ELITT) system (Sharplan Laser Ltd., Needham, Massachusetts, USA) consists of three diffuser laser fibres, joined distally by semi-rigid plastic arms. Proximally they are attached to a hand piece. The system is passed through the cervix, dilated to 8mm, and once in the uterine cavity the system is activated by opening the 'arms' which cause the fibres to lie in contact with the lateral parts of the cavity. A 20 W diode laser is activated for 7 minutes. Data from women followed-up for at least a year showed 63% - 71% amenorrhoea rates (Donnez et al 1996; Donnez et al 2000). The rate of amenorrhoea or severe hypomenorrhoea was greater than 90% (Donnez et al 2000).

11.2.5.3 Diathermy.

The Novacept device (Novasure, Palo Alto, California, USA) uses bipolar energy and a distendable wire mesh to impart the energy (Cooper 1998). The endometrium is drawn into the energy field by suction. The treatment time is automatic, based on impedance in the tissues, but usually lasts between one and two minutes. There are, however, no published data using this device.

The Vestablate system (Vesta Medical Inc, Mountail View, California) is a multielectrode intrauterine balloon device. The controller regulates energy to each of the 12 plates by monitoring the temperature at each electrode. The treatment cycle is set at 75°C. Treatment lasts 4 minutes after a one minute 'warm-up'. Results compared with TCRE are of amenorrhoea rates of 31% compared with 40% at 1 year.

11.2.5.4 *Radiofrequency and microwave.*

These energy sources are very similar, but must not be confused. Radiofrequency endometrial ablation (RaFEA) uses a frequency of 27.12 MHz. Thermal damage is caused by the forced oscillation of charged particles in the endometrium caused by the device, passed into the cavity. The endometrial surface temperature is raised to approximately 65°C (Phipps et al 1990a; Phipps et al 1990b). The procedure takes 20 minutes, with the probe

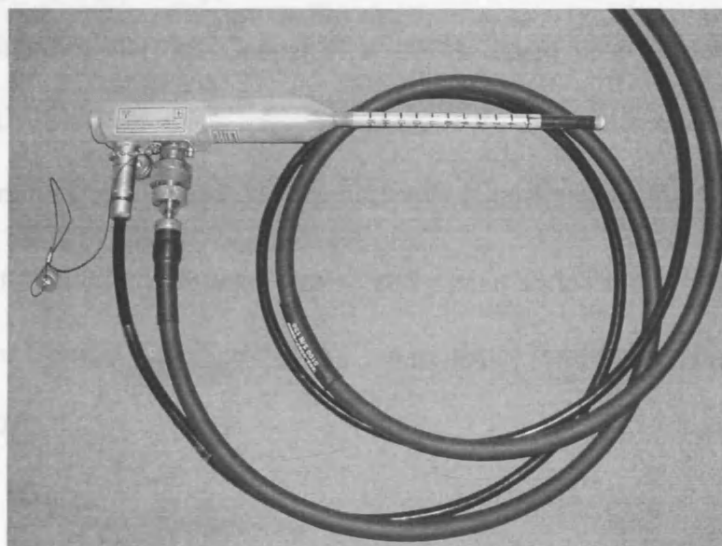


Figure 11.7: Microwave ablation – handset.

being rotated to ensure an even destruction.

Microwave endometrial ablation (MEA) at 9.2 GHz destroys the endometrium to a depth of 6 mm (Sharp et al 1995). The commercial system (Microsulis Ltd., Waterlooville, UK) requires the cervix to be dilated to 8 mm and by way of thermocouples, the temperature of the endometrium is monitored (figure 11.2). The operator maintains a steady 'back-and-forth' movement within the cavity, slowly withdrawing the device. The system screen records temperature. The operating temperature is 70 - 80°C. The system alarms at 85°C, and shuts off at 90°C. An advantage of this system is that if the initial temperature rise fails, suggesting an uterine perforation, the system will also shut down. The procedure takes a few minutes only.

With power levels of 30 W, and energies approaching 10 kJ, 5 - 6 mm deep necrosis can be achieved. Short-term data comparing this technique with TCRE is encouraging giving 80% satisfaction and 57% amenorrhoea at 6 months (Sharp et al 1995; Cooper et al 1998). Three year results compared with TCRE have an amenorrhoea rate of 40% for both modalities with satisfaction rates of 77% and 75% respectively (Cooper et al 1999).

The use of Microwave ablation has recently been supported by the National Institute for Clinical Excellence (NICE) in guidance published in August 2003 (NICE 2003).

11.2.5.5 Cryoablation.

The First Option system (CryoGen, San Diego, California) uses carbon dioxide, freon gas and nitrous oxide gas under pressure to freeze the tip of a 9 mm probe. The probe is similar in shape to a hegar dilator. The tip of the

probe reaches temperatures of -55°C , which causes cell death to a depth of 3.2-7.2mm when in direct contact with the endometrium (Kremer et al 2000). Treatment times last 2 minutes. At 12 month follow-up compared to rollerball, there are satisfaction rates of 88% for both treatments; amenorrhoea rates of 28% and 52% respectively (Dobak et al 2000).

With the other techniques available as discussed, we can consider the advantages and disadvantages of photodynamic therapy.

11.2.6 Photodynamic therapy

Unlike other methods of local injury, for example thermal coagulation with a laser or diathermy, or ionising radiation, the biological effects on tissues with PDT is such that after healing, there is no resultant weakening of the underlying strength of the collagen in these tissues (Barr et al 1987; Pope and Bown 1991; Pope et al 1991). PDT does not cause necrosis in the underlying collagen matrix of the target tissue. This makes PDT a very attractive option for endometrial destruction. However it must compete with the “successes and patient satisfaction of the other devices and techniques .

Kennedy and Pottier showed that using ALA intravenously (i.v.) in mice resulted in high levels of the photosensitiser PPIX in the endometrium. They also showed that there were much lower levels in the myometrium (Kennedy and Pottier 1992). Judd et al gave ALA i.v. and showed PPIX fluorescence nine times higher in the endometrium as the myometrium at 3 hours (Judd et al 1992).

Chapman et al gave photofrin either i.v. (7mg/kg), intraperitoneally (i.p.) (7mg/kg), or at laparotomy into the uterine horns (i.u.) (0.7mg/kg) of rats. She

showed that endometrial Photofrin fluorescence was highest in those rats given i.u. ALA, despite the lower dose.

Chapman also examined the effects of oestrogen on uptake of photofrin on rats after i.u. instillation. The levels were highest in the group given oestrogen alone, compared with GnRHa suppression, GnRHa suppression with oestrogen or no oestrogen (Chapman 1993).

Photodynamic therapy following topical administration into the uterine horns followed. Yang et al showed that there was a difference in pregnancy rates between the two horns in rats that underwent uterine PDT 3 hours after pre-treatment with ALA injected into one uterine horn. There was no difference in implantation rates in those rats bred at 10 days using ALA compared to saline in the non-PDT group (76% implantation vs. 92%). However in the light-treated group there was a difference in implantation rates of 3.8% compared with 100%). He also showed that the endometrial destruction caused by ALA-induced PDT was lasting. Rats bred at 60 days after PDT had 16.7% implantations in the treated horns compared with 100% in the untreated horns (Yang et al 1993).

Steiner et al showed that after ALA-induced PDT there was destruction of the endometrium and atrophy of the horns in the treated side. Also that reproductive performance was significantly worse on the treated side. There were a mean of 0.4 sacs on the treated side compared with 8.9 sacs on the untreated side). He showed that the peak levels of PPIX were reached 3-6 hours after topical administration (Steiner et al 1996).

Pius Wyss from the Beckman Laser Institute in California performed a number of experiments. His initial set of experiments was designed to determine optimal parameters for PDT of the endometrium. He used rats and rabbits to show peak PPIX fluorescence at 3 hours in both animal models with uterine instillation of ALA, with much higher levels in the endometrium compared with the myometrium. Peak levels were analysed with a variation of dose of ALA. This resulted in the highest peak at 200mg/ml. He altered the pH of the solution from pH 1.6 - 2.2 (unbuffered); to pH 5.5 and showed no significant variations in levels at the different pH (Wyss et al 1994).

After ALA administration into monkey uteri PPIX fluorescence was noted at 4-5 hours (Yang et al 1996). The monkeys were noted to be able to tolerate high doses of ALA with no ill-effects; the only anomaly was a high aspartate aminotransferase level in serum samples at 24 hours (Yang et al 1996).

Michael Gannon in Leeds undertook a series of experiments on human uteri in vivo and in vitro. In the in vitro study, 4 uteri had the uterine arteries cannulated and were perfused with 0.9% saline at 37.5⁰C, ALA was instilled into the cavities. He showed a ten-fold increase in PPIX fluorescence in the endometrium compared with the myometrium. The in vivo study involved 8 women who were undergoing hysterectomy. They had varying amounts of ALA instilled into the uterine cavity 2-5 hours before the hysterectomy. Again, levels of PPIX fluorescence were some nine times higher in the endometrium than the myometrium. In both sets of experiments the fluorescence was noted throughout the endometrium including the deep glands (Gannon 1995).

ALA solution has been instilled into the uterine cavities of 27 women by Fehr et al (1996). He showed peak fluorescence to occur at between 4 and 8 hours; this was 48 times higher than the myometrium, and suggested that selective PDT causing endometrial destruction would be possible.

Human PDT using ALA has been performed (Gannon et al 1998). Gannon has treated over 30 women but the results are highly variable, although there have been no adverse events (Gannon personal communication).

Topical application of ALA in the uterus allows lower doses of drug to be used. This in turn results in reduced systemic levels and therefore may eliminate systemic side-effects, notably skin photosensitivity (Dougherty et al 1990; Razum et al 1987; Roberts et al 1995). Current clinical experience of PDT induced with topical ALA, has had limited success (Gannon et al 1998) we presume, partly due to incomplete illumination of the uterine cavity, and partly due to insufficient levels of PPIX being present throughout the endometrium. By adding CP94 and therefore chelating iron locally, the conversion of the photoactive protoporphyrin IX (PPIX) to haem may be reduced, leading to higher and more persistently higher local concentrations of PPIX in the endometrium. Our aim was to ascertain the optimal conditions for endometrial PDT with ALA prior to embarking on clinical trials.

The two methods of increasing the PDT effect in the human uterus are to increase photosensitiser concentration, or increase the amount of light. Unfortunately the human endometrial cavity is a non-uniform cavity in the shape of a flattened triangle. The light distribution within the cavity is therefore non-uniform and somewhat unpredictable. Our study has shown that, in the animal model, higher levels of photosensitiser are present in the

endometrium by using an iron chelator CP94 compared to ALA alone. Topical administration of ALA with CP94 led to a dramatic increase in the PPIX fluorescence in the endometrium. The increase was more than doubled in the superficial endometrium, 1.7 times increased in the deep endometrial glands and was three times less in the myometrium. Overall the superficial endometrium had levels of PPIX almost 30 times higher than the myometrium.

We have shown an improved and long lasting PDT effect in the animal model. We can now take these techniques to the clinical setting.

In order to increase the light dose, a balloon device can be utilised. The selective destruction is of paramount importance, as the myometrium varies in thickness throughout the uterus. The area where regeneration is most likely to occur is near the ostia and it is here that the myometrium is at it's most thin. The selectivity of PDT is therefore very desirable.

11.2.6.1 *Clinical studies.*

The endometrium lends itself to topical administration of photosensitiser. The concentrations of drug are much higher than could be achieved by the oral or IV route, because of the systemic side-effects.

It has to be stated that there may be no inter-species similarity, and the peak photosensitivity will have to be fully investigated in the human uterus. The difficulties will not end here, indeed the stage of the menstrual cycle could have a very large part to play in the success of treatment.

Light distribution within the uterine cavity has proved to be a more demanding problem. The application of a translucent balloon into a semi-reflective

slightly distended cavity has resulted in good overall illumination in a normal uterus. The results should also have been reproducible. Our results appear acceptable in terms of light dosimetry. If the drug can be instilled in an acceptable fashion then there could be successful and long lasting endometrial destruction in the human.

11.3 Future development.

The problem of low levels of PPIX concentration in the endometrium has been overcome by this study, by the coadministration of CP94. Light dosimetry in the rabbit has not been an issue, as the rabbit uterine horns are essentially cylinders. The human endometrial cavity posed greater problems. This appears to have been successfully tackled with our balloon device.

The future development of ALA PDT for endometrial destruction in menorrhagia should now be aimed at specific areas.

The pharmacokinetics of ALA absorption within the uterine cavity has to be addressed. Although Wyss showed that peak absorption with rats and rabbits was around 4 hours (Wyss et al 1996), there can be no such presumption in the human. As discussed previously, the oestrogen status of the uterus has an impact on drug absorption and PDT effects, therefore a similar investigation will have to be performed in women. Garry and others have shown that pre-treatment of the endometrium prior to endometrial ablation has improved the effectiveness, whether this is merely a curettage prior to the procedure, or some months treatment with GnRHa analogues (Garry et al 1999).

Distension of the uterine cavity either with a liquid or by a balloon device appears the way forward. The limitations of distension causing pain are not

borne out with the use of the newer endometrial ablation balloons - used under local anaesthetic and inflated with more than 15 ml saline to a pressure of over diastolic blood pressure with little problem. PDT does depend on oxygen in the tissues and therefore any distension should be kept to a minimum to prevent hypoxia in the tissues.

Use of devices with multiple diffuser fibres seems an avenue that could also be investigated.

Other areas of gynaecology could also benefit from PDT. The treatment of endometriosis is promising. The idea of selective uptake of photosensitiser by active endometriosis in the peritoneal cavity with subsequent light application at laparoscopy causing specific ablation of the endometriosis sparing healthy tissue is an exciting concept.

The use of phthalocyanines in endometrial ablation has appeal. The longer wavelength needed to activate these drugs, means that the light dose penetrates the tissues to a greater degree. This should lead to greater and more sustained destruction of the endometrium. However, destruction must be limited to the endometrium.

The coupling of photosensitisers to antibodies or viral vectors to target tissues very specifically is exciting and could have specific uses in gynaecology (Schmidt et al 1992).

11.4 Limitations of PDT in the treatment of menorrhagia.

The results of other workers showing peak fluorescence at 3 and 4 hours after administration of ALA would have meant that ALA-PDT was quite an

attractive proposition (Wyss et al 1996). A woman could attend the hospital ward in the morning. She could have a small intrauterine catheter placed transcervically. A 1 ml balloon would prevent spillage and keep the catheter in place. She would then be free for two or three hours, would return and have a short treatment in the examination chair, with little or no discomfort.

Unfortunately our results are at odds with most workers. Interestingly Wyss found peak fluorescence to be between 4 and 8 hours. He then treated at four hours. Our results show a small peak at three to four hours and a second peak at seven to eight hours. However we found a much greater 7 hour peak with the addition of CP94.

Administration to a woman of the drug 7 hours prior to treatment is much less user friendly. The treatment time then necessitates either an overnight stay or a long all day stay. Both users and providers find a short 'one stop' approach much more attractive.

11.5 Summary of the future developments in PDT for endometrial destruction.

Work is continuing to further investigation PDT for endometrial destruction in the clinical setting.

1. Initially ALA and CP94 will be investigated by giving the drugs topically to the uterus of women about to undergo hysterectomy, in a similar manner to our animal experiments.
2. When the best drug dose is known a small part of the endometrium will be treated at different time scales after drug administration three days prior to hysterectomy.

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3. When the correct light dose is known, the whole endometrium will be treated three days prior to hysterectomy.
 4. The next step would be to investigate long-term success – measured over a few weeks prior to hysterectomy.
 5. Then we would investigate long-term success without subsequent hysterectomy.
 6. Finally the aim is to compare PDT with other MAS treatments for menorrhagia in a RCT.

11.6 Conclusion.

Successful photodynamic ablation of the endometrium can be carried out with ALA. This thesis has shown that it is possible to substantially increase the PPIX levels in the glandular elements of the endometrium and also to increase the destruction after PDT by the addition of a novel iron-chelating agent CP94. This destruction is not at the expense of myometrial destruction as the PPIX levels within the myometrium remain low. This is important from a safety point of view as it prevents the possibility of collateral (extra-uterine) tissue damage. Selectivity and increased tissue damage is the key to the addition of CP94.

There are problems that have been brought up by this thesis. The light distribution within the cavity will be of huge importance for successful clinical outcomes. Once the light distribution has been sorted out, successful PDT of the endometrium could become a reality. However, the simplicity of this technique is marred by the potential hindrance of having to pre-treat the endometrium – possibly 7-8 hours prior to treatment. This is of considerable

inconvenience to the women – and the clinical staff. It may also be that ALA and CP94 compares badly with phthalocyanines with respect to long-term endometrial ablation, because of the possibility of deeper destruction with the latter. These are exciting areas to study in the future. There may be an advantage in pursuing the phthalocyanines, with the longer wavelength activation - and therefore deeper light penetration - as photosensitisers.

Work is currently ongoing at our centre, continuing the light distribution work and investigating the phthalocyanines.

REFERENCES

- Abberton KM, Taylor NH, Healy DL, Rogers PA. Vascular smooth muscle cell proliferation in arterioles of the human endometrium. *Human Reproduction* 1999; 14: 1072-9.
- Adelantado JM, Rees MC, Lopez Bernal A, Turnbull AC. Increased uterine prostaglandin E receptors in menorrhagic women. *British Journal of Obstetrics & Gynaecology* 1988; 95: 162-5.
- Adelantado JM, Lopez Bernal A, Turnbull AC. Topographical distribution of prostaglandin E receptors in human myometrium. *British Journal of Obstetrics & Gynaecology* 1988; 95: 348-53.
- Agnelli G, Gresele P, De Cunto M et al. Tranexamic acid, intrauterine contraceptive devices and fatal cerebral thrombosis. Case report. *British Journal of Obstetrics and Gynaecology* 1982; 89: 681-682.
- al-Azzawi F. Hysteroscopy or ultrasound? *Curr Opin Obstet Gynecol* 1996; 8: 246-9.
- Alecozay AA, Harper MJ, Schenken RS, Hanahan DJ. Paracrine interactions between platelet-activating factor and prostaglandins in hormonally-treated human luteal phase endometrium in vitro. *J Reprod Fertil.* 1991; 91:301-12.
- Alexander AD, Naji AA, Pinion SB et al. Randomised trial comparing hysterectomy with endometrial ablation for dysfunctional uterine bleeding: psychiatric and psychosocial aspects. *BMJ* 1996; 312: 280-284.
- Amso NN, Stabinsky SA, McFaul P, Blanc B, Pendley L, Neuwirth R. Uterine thermal balloon therapy for the treatment of menorrhagia: the first 300 patients from a multi-centre study. *International Collaborative Uterine Thermal Balloon Working Group. British Journal of Obstetrics & Gynaecology* 1988; 105(5): 517-23.
- Anath J. Hysterectomy and depression. *Obstet Gynecol* 1978; 52: 724-730.
- Andersch B, Milsom I, Rybo G. An objective evaluation of flurbiprofen and tranexamic acid in the treatment of idiopathic menorrhagia. *Acta Obstetricia et Gynecologica Scandinavica* 1988; 67; 645-8.
- Anderson AB, Haynes PJ, Guillebaud J, Turnbull AC. Reduction of menstrual blood-loss by prostaglandin-synthetase inhibitors. *Lancet* 1976; 1; 774-6.
- Anderson MC. Treatment of CIN with the CO₂ laser: report of 543 patients. *Obstet Gynecol.* 1982; 59: 720-725.
- Andersson JK, Rybo G. Levonorgestrel-releasing intrauterine device in the treatment of menorrhagia. *British Journal of Obstetrics & Gynaecology* 1990; 97; 690-4.
- Andersson L. Fibrinolysis and its control. *South African Medical Journal* 1972; 46: 182-5.
- Anonymous. Surgical management of menorrhagia. *Drug & Therapeutics Bulletin* 1994; 32: 70-2.
- Anonymous. A Scottish audit of hysteroscopic surgery for menorrhagia: complications and follow up. *Scottish Hysteroscopy Audit Group. British Journal of Obstetrics & Gynaecology* 1995; 102; 249-54.
- Anonymous. National Confidential Enquiry into Perioperative Deaths 1996-1997. London: HMSO, 1998.
- Ascher SM, Johnson JC, Barnes WA, Bae CJ, Patt RH, Zeman RK. MR imaging appearance of the uterus in postmenopausal women receiving tamoxifen therapy for breast cancer: histopathologic correlation. *Radiology.* 1996; 200:105-10.

-
- Asherman JG. Amenorrhoea traumatica (atretica). *J Obstet Gynaecol Brit Empire* 1948; 100: 161-164.
- Backman T, Huhtala S, Blom T, Luoto R, Rauramo I, Koskenvuo M. Length of use and symptoms associated with premature removal of the levonorgestrel intrauterine system: a nation-wide study of 17,360 users. *British Journal of Obstetrics & Gynaecology* 2000; 107: 335-339.
- Baggish MS and Dorsy JH. CO₂ laser for the treatment of vulvar carcinoma in situ. *Obstet Gynecol* 1981; 57: 371-5.
- Baird DT, Cameron ST, Critchley HO, Drudy TA, Howe A, Jones RL, Lea RG, Kelly RW. Prostaglandins and menstruation. *Eur J Obstet Gynecol Reprod Biol.* 1996;70:15-7.
- Baldwin RM, Whalley PJ, Pritchard JA. Measurement of menstrual blood loss. *Am L Obstet Gynecol* 1961; 81: 739-742.
- Barer AP and Fowler FM. The blood loss during normal menstruation. *Am J Obstet Gynecol* 1938; 35: 839-41.
- Barglow P, Gunther MS, Johnson A, Meltzer HJ. Hysterectomy and tubal ligation: a psychiatric comparison. *Obstet Gynecol.* 1965; 25: 520-7.
- Barr H, Tralau CJ, Boulos PB, MacRobert AJ, Tilly R, & Bown SG. The contrasting mechanisms of colonic collagen damage between photodynamic therapy and thermal injury. *Photochem Photobiol*, 1987; 46: 795-800.
- Barrington JW, Bowen-Simpkins P. The levonorgestrel intrauterine system in the management of menorrhagia. *British Journal of Obstetrics & Gynaecology* 1997; 104: 614-6.
- Basov NG, Danilychev VA, Popov YUM, Khodkevitch DD. Laser operating in the vacuum region of the spectrum by excitation of liquid xenon with electron beam. *JETP Lett* 1970; 12: 329-333.
- Belsey EM, Farley TM. The analysis of menstrual bleeding patterns: a review. *Contraception* 1988; 38: 129-56.
- Berenbaum MC, Akande SL, Bonnett R, Kaur H, Ioannou S, White RD, Winfield UJ. meso-Tetra(hydroxyphenyl)porphyrins, a new class of potent tumour photosensitisers with favourable selectivity. *Br J Cancer.* 1986; 54:717-25.
- Berg, K., Anholt, H., Bech, O. & Moan, J. The influence of iron chelators on the accumulation of protoporphyrin IX in 5-aminolaevulinic acid treated cells. *Br J Cancer*, 1996; 688-97.
- Berget A, Andreasson B, Bock JE, Bostofte E, Hebjorn S, Isager-Sally L, Philipsen T, Schantz A, Weber T. Outpatient treatment of cervical intra-epithelial neoplasia. The CO₂ laser versus cryotherapy, a randomized trial. *Acta Obstet Gynecol Scand.* 1987; 66: 531-6.
- Belsey EM, Farley TM. The analysis of menstrual bleeding patterns: a review. *Contraception* 1988; 38: 129-56.
- Bishop PM and Cabral deAlmeida JC. Treatment of functional menstrual disorders with norethisterone. *Br Med J.* 1960; 9: 5179: 1103-5.
- Bloomfield M. Sacred books of the East; Hymns of Atharva-Veda Vol XLII. Clarendon Press, Oxford, 1897 (cited in Fitzpatrick and Pathak 1958).
- Bombieri L, Hogston P. Endometrial carcinoma with an endometrial thickness of 3.2mm on vaginal ultrasound. *J Ultrasound Med* 1986; 5: 445-452.
- Bongers MY, Mol BW, Dijkhuizen FP, Broilmann HA. Is balloon ablation as effective as electroresection in the treatment of menorrhagia? *J Laparoend Adv Surg Tech* 2000; 10: 85-92.
-

-
- Bonnar J and Sheppard BL. Treatment of menorrhagia during menstruation: randomised controlled trial of ethamsylate, mefenamic acid and tranexamic acid. *BMJ* 1996; 313: 579-582.
- Bonnett, R., White, R., Winfield, U. & Berenbaum, M.C. (1989). Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as photosensitisers. *Biochem J.* 1989; 261: 277-280.
- Bonnett R. Photosensitisers of the porphyrin and phthalocyanine series for photodynamic therapy. *Chem Soc Rev* 1995; 24: 19-23.
- Bonnett R. Photodynamic therapy in Historical perspective. *Rev Contemp Pharmacother* 1999; 10: 1-17.
- Bourne TH, Campbell S, Whitehead M, Royston P Steer C, Collins W. Detection of endometrial cancer in postmenopausal women by transvaginal ultrasonography and colour flow imaging. *BMJ* 1990; 301:369.
- Bourne TH, Lawton F, Leather A, Granberg S, Campbell S, Collins WP. Use of intracavity saline instillation and transvaginal ultrasonography to detect tamoxifen-associated endometrial polyps. *Ultrasound Obstet Gynecol.* 1994; 4: 73-5.
- Bown S, Traulau C, Coleridge-Smith P, Akdemir D, Wieman T. Photodynamic therapy with porphyrin and phthalocyanine sensitisation: quantitative studies in normal rat liver. *Br J Cancer*, 1986; 54: 43-52.
- Bown SG. Photodynamic therapy - basic principles. In *Lasers in Gastroenterology - International Experiences and Trends*. Reiman JF and Ell C (Eds); 1989: pp 85-92. Thieme: Stuttgart.
- Bown SG. Photodynamic therapy to scientists and clinicians--one world or two? *J Photochem Photobiol B*, 1990; 6: 1-12.
- Bradley LD, Widrich T. State-of-the-art flexible hysteroscopy for office gynecologic evaluation. *J Am Assoc Gynecol Laparosc.* 1995; 2:263-7.
- Bridgman SA, Dunn KM. Has endometrial ablation replaced hysterectomy for the treatment of dysfunctional; uterine bleeding? National figures. *British Journal of Obstetrics & Gynaecology* 2000; 107: 531-534.
- Brittenham GM. Development of iron-chelating agents for clinical use. *Blood* 1992; 80: 569-574.
- Buttram VC Jr, Reiter RC. Uterine leiomyomata: etiology, symptomatology, and management. *Fertility & Sterility* 1981; 36: 433-45.
- Cacciatore B, Ramsay T, Lehtovirta P, Ylostalo P. Transvaginal sonography and hysteroscopy in postmenopausal bleeding. *Acta Obstet Gynecol Scand.* 1994; 73: 413-6.
- Cameron IT, Leask R, Kelly RW, Baird DT. The effects of danazol, mefenamic acid, norethisterone and a progesterone-impregnated coil on endometrial prostaglandin concentrations in women with menorrhagia. *Prostaglandins.* 1987; 34: 99-110.
- Cameron IT. Dysfunctional uterine bleeding. *Baillieres Clinical Obstetrics & Gynaecology* 1989; 3: 315-27.
- Campbell S, Bourne T, Crayford T, Pittrof R. The early detection and assessment of endometrial cancer by transvaginal colour Doppler ultrasonography. *Eur J Obstet Gynecol Reprod Biol.* 1993; 49:44-5.
- Carruth J. Lasers in medicine and surgery. *J Med Eng Technol*, 1984; 8: 161-7.
- Casslen B, Astedt B. Occurrence of both urokinase and tissue plasminogen activator in the human endometrium. *Contraception* 1983; 28:553-64.
-

-
- Casslen B. Inhibitors of trypsin, chymotrypsin and elastase in human uterine fluid. *Acta Obstetrica et Gynecologica Scandinavica* 1986; 65: 121-4.
- Casslen B, Andersson A, Nilsson IM, Astedt B. Hormonal regulation of the release of plasminogen activators and of a specific activator inhibitor from endometrial tissue in culture. *Proceedings of the Society for Experimental Biology & Medicine* 1986; 182: 419-24.
- Casslen B, Nordengren J, Gustavsson B, Nilbert M, Lund LR. Progesterone stimulates degradation of urokinase plasminogen activator (u-PA) in endometrial stromal cells by increasing its inhibitor and surface expression of the u-PA receptor. *Journal of Clinical Endocrinology & Metabolism* 1995; 80: 2776-84.
- Chamberlain G and Foulkes J. Menstruation after laparoscopic sterilisation. *Proc R Soc Med* 1976; 69: 825.
- Chamberlain G, Freeman R, Price F, Kennedy A, Green D, Eve L. A comparative study of ethamsylate and mefenamic acid in dysfunctional uterine bleeding. *British Journal of Obstetrics & Gynaecology* 1991; 98: 707-11.
- Chang SC, MacRobert AJ, Porter JB, Bown SG. The efficacy of an iron chelator (CP94) in increasing cellular protoporphyrin IX following intravesical 5-aminolevulinic acid administration: an in vivo study. *J Photochem Photobiol B: Biol* 1997; 38: 114-122.
- Chang, S., MacRobert, A., Porter, J. & Bown, S. (1994). Application of 1,2-diethyl-3-hydroxypyridine-4-one to enhance tissue selectivity for photodynamic therapy of the bladder. In *International Conference on Photodynamic Therapy and Medical Laser Application*, Spinelli, P., Dal Fante, M. & Marchesini, R. (eds), Vol. 2371. pp. 322-326. Elsevier Science Publishers B.V.: Milan.
- Chang SC, MacRobert AJ, Bown SG. Biodistribution Of Protoporphyrin-IX In Rat Urinary-Bladder After Intravesical Instillation Of 5-Aminolevulinic Acid. *Journal Of Urology* 1996; 155: 1744-1748.
- Chapman JA, Tadir Y, Tromberg BJ, Yu K, Manetta A, Sun CH, Berns MW. Effect of administration route and estrogen manipulation on endometrial uptake of Photofrin. *Am J Obstet Gynecol* 1993; 168: 685-692.
- Charnock-Jones DS, Sharkey AM, Jagers DC, Yoo HJ, Heap RB, Smith SK. In-vivo gene transfer to the uterine endometrium. *Hum Reprod* 1997; 12: 17-20.
- Cheyne GA and Shepherd MM. Comparison of chemical and atomic absorption method for estimating menstrual blood loss. *J Med Lab Technol* 1970; 27: 350-360.
- Chimbira TH, Cope E, Anderson ABM, Bolton FG. The effect of danazol on menorrhagia, coagulation mechanisms, haematological indices and body weight. *British Journal of Obstetrics & Gynaecology* 1979; 86: 46-50.
- Chimbira TH, Anderson AB, Naish C, Cope E, Turnbull AC. Reduction of menstrual blood loss by danazol in unexplained menorrhagia: lack of effect of placebo. *British Journal of Obstetrics & Gynaecology* 1980; 87: 1152-8.
- Christiaens GC, Sixma JJ, Haspels AA. Haemostasis in menstrual endometrium in the presence of an intrauterine device. *British Journal of Obstetrics & Gynaecology* 1981; 88: 825-37.
- Christiaens GC, Sixma JJ, Haspels AA. Hemostasis in menstrual endometrium: a review. *Obstetrical & Gynecological Survey* 1982; 37: 281
- Cicinelli E, Romano F, Anastasio PS, Blasi N, Parisi C. Sonohysterography versus hysteroscopy in the diagnosis of endouterine polyps. *Gynecol Obstet Invest* 1994; 38: 266-271.
- Claessens EA and Cowell CA. Acute adolescent menorrhagia. *Am L Obstet Gynecol* 1982; 139: 277-280.
-

-
- Cole S, Billewicz W, Thomson A. Sources in variation of menstrual blood loss. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1971; 78: 933-939.
- Cole SK, Clarkson AR. Menstrual blood loss and fibrin degradation products. *British Medical Journal* 1972; 1; 78-9.
- Coleman ML, McCowan L, Farquhar C. The levonorgestrel-releasing intrauterine device: a wider role than contraception. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 1997; 37: 195-201.
- Conoscenti G, Meir YJ, Fiscer-Tamparo L, Maieron A, Natale R, D'Ottavio G, Rustico M, Mandruzzato G. Endometrial assessment by transvaginal sonography and histological findings after D & C in women with postmenopausal bleeding. *Ultraound Obstet Gynaecol.* 1995; 6: 108-115.
- Cooper KG, Parkin DE, Garratt AM, Grant AM. A randomised comparison of medical and hysteroscopic management in women consulting a gynaecologist for treatment of heavy menstrual loss. *British Journal of Obstetrics & Gynaecology* 1997; 104: 1360-6.
- Cooper KG, Bain C, Parkin DE. A randomised trial comparing microwave endometrial ablation with transcervical resection of the endometrium. *British Journal of Obstetrics & Gynaecology* 1998; 105: 25.
- Cooper KG, Parkin DE, Garratt AM, Grant AM. Two-year follow up of women randomized to medical management or transcervical resection of the endometrium for heavy menstrual loss: clinical and quality of life outcomes. *British Journal of Obstetrics & Gynaecology* 1999; 106: 258-265.
- Copperman AB, DeCherney AH, Olive DL. A case of endometrial cancer following endometrial ablation for dys-functional uterine bleeding. *Obstet Gynecol* 1993; 82: 640-642
- Corson SL. A multicenter evaluation of endometrial ablation by Hydro ThermAblator and rollerball for treatment of menorrhagia. *J Am Assoc Gynecol Laparosc.* 2001; 8: 359-67.
- Corson SL, Brill AI, Brooks PG, Cooper JM, Indman PD, Liu JH, Soderstrom RM, Vancaillie TG. One-year results of the vesta system for endometrial ablation. *J Am Assoc Gynecol Laparosc.* 2000;7:489-97
- Coulter A, Noone A, Goldacre N. General practitioners' referrals to specialist outpatient clinics. I. Why general practitioners refer patients to specialist outpatient clinics. *BMJ* 1989; 299: 304-6.
- Coulter A, Bradlow J, Agass M, Martin-Bates C, Tulloch A. Outcomes of referrals to gynaecology outpatient clinics for menstrual problems: an audit of general practice records. *British Journal of Obstetrics & Gynaecology* 1991; 98: 789-96.
- Coulter A, Kelland J, Peto V, Rees MC. Treating menorrhagia in primary care. An overview of drug trials and a survey of prescribing practice. *International Journal of Technology Assessment in Health Care* 1995; 11; 456-71.
- Cremer RJ, Perryman PW, Richards DH. Influence of light on the hyperbilirubinaemia of infants. *Lancet.* 1958 May 24;1:1094-7
- Curnow A, McIlroy BW, Postle-Hacon MJ, Porter JB, MacRobert AJ, Bown SG. Enhancement of 5-aminolaevulinic acid-induced photodynamic therapy in normal rat colon using hydroxypyridinone iron-chelating agents. *Br J Cancer* 1998; 78: 1278-1282.
- Curnow A. Photodynamic Therapy with 5-Aminolaevulinic Acid - Techniques for Enhancement. Ph.D. Thesis, University of London 1999; pp 37-44.
- Daniell MD and Hill JS. A history of photodynamic therapy. *Aust NZ J Surg* 1991; 61: 340-348.
-

-
- Datey S, Gaur LN, Saxena BN. Vaginal bleeding patterns of women using different contraceptive methods (implants, injectables, IUDs, oral pills)--an Indian experience. An ICMR Task Force Study. Indian Council of Medical Research. Contraception 1995; 51: 155-65.
- Dawood MY, Lewis V, Ramos J. Cortical and trabecular bone mineral content in women with endometriosis: effect of gonadotrophin-releasing hormone agonist and danazol. Fertil Steril 1989; 52: 21-26.
- Davey DA. Dysfunctional uterine bleeding. In: Dewhurst's Textbook of Obstetrics and gynaecology for postgraduates. Whitfield CR (ed.), Fifth edition: 1995; 590-608. Blackwell Science Ltd. Oxford.
- DeCherney A, Polan ML. Hysteroscopic management of intrauterine lesions and intractable uterine bleeding. Obstet Gynecol 1983; 61: 392-397.
- DeCherney AH, Diamond MP, Lavy G, Polan ML. Endometrial ablation for intractable uterine bleeding. Obstetrics and Gynaecology 1987; 70: 668-670.
- Deprest JA, Munro MG, Koninckx PR. Review on laparoscopic hysterectomy. Zentralblatt für Gynäkologie 1995; 117(12): 645-651. (cited in Hill 1997).
- Dicker RC, Greenspan JR, Strauss LT, Cowart MR, Scally MJ, Peterson HB, DeStefano F, Rubin GL, Ory HW. Complications of abdominal and vaginal hysterectomy among women of reproductive age in the United States. The Collaborative Review of Sterilization. American Journal of Obstetrics & Gynecology 1982; 144: 841-8.
- Dijkhuizen F, Brölmann H, Potters A, Bongers M, Heintz A. Accuracy of transvaginal ultrasonography in the diagnosis of endometrial abnormalities. Obstet Gynecol 1996; 87: 345-349.
- Dobak JD, Ryba E, Kovalcheck S. A new closed-loop cryosurgical device for endometrial ablation. J Am Assoc Gynecol Laparosc. 2000;7:245-9.
- Dockeray CJ, Sheppard BL, Bonnar J. Comparison between mefenamic acid and danazol in the treatment of established menorrhagia. British Journal of Obstetrics & Gynaecology 1989; 96: 840-4.
- Donnez J, Polet R, Rabinovitz R, Squifflet J, Nisolle M. Endometrial laser intrauterine thermotherapy: the first series of 100 patients observed for 1 year. Fertil Steril 2000; 74(4): 791-6.
- Donnez, J., R. Polet, et al. (1996). Endometrial laser interstitial hyperthermy: a potential modality for endometrial ablation. Obstetrics and Gynecology 87: 459-464.
- Dougherty TJ, Kaufman JE, Goldfarb A, Weishaupt KR, Boyle D, Mittleman A. Photoradiation therapy for the treatment of malignant tumors. Cancer Res 1978; 38(8): 2628-35.
- Dougherty, T., Cooper, M. & Mang, T. Cutaneous phototoxic occurrences in patients receiving Photofrin. Lasers Surg Med 1990; 10:485-88.
- Dougherty T, Grindey G, Fiel R, Weishaupt K. & Boyle D. Photoradiation therapy II. Cure of animal tumours with hematoporphyrin and light. J Nat Cancer Inst, 1975; 55, 117.
- Dougherty TJ, Kaufman JE et al. Photoradiation therapy for the treatment of malignant tumors. Cancer Res 1978; 38: 2628-35.
- Dougherty T & Marcus S. Photodynamic therapy. Eur J Cancer 1992; 28, 1734-42.
- DTB. Levonorgestrel intra-uterine system for menorrhagia. Drug and Therapeutic Bulletin. 2001; 39: 85-88.
- DTB. Frameless intra-uterine contraceptive device (Gynefix). Drug and Therapeutic Bulletin. 2002; 40: 21-2.
-

-
- Dudiak CM, Turner DA, Patel SK, Archie JT, Silver B, Norusis M. Uterine leiomyomas in the infertile patient: preoperative localization with MR imaging versus US and hysterosalpingography. *Radiology*. 1988; 167: 627-30.
- Elfsson B, Wallin I, Eksborg S, Rudaeus K, Ros AM, Ehrsson H. Stability of 5-aminolevulinic acid in aqueous solution. *European Journal of Pharmaceutical Sciences* 1998; 7: 87-91.
- El- Mofty AM. Observations on the use of Ammi majus Linn. In vitiligo. *Br J Dermatol*. 1952; 64: 431-41.
- El-Toukhy TA, Hefni M, Davies A, Mahadevan S. The effect of different types of hysterectomy on urinary and sexual function: a prospective study. *J Obstet Gynaecol*. 2004; 24(4):420-5.
- Emanuel MH, Verdel MJ, Wamsteker K, Lammes FB. A prospective comparison of transvaginal ultrasonography and diagnostic hysteroscopy in the evaluation of patients with abnormal uterine bleeding: clinical implications. *American Journal of Obstetrics & Gynecology* 1995; 172: 47-52.
- Epstein JH. Phototherapy and photochemotherapy. *N Engl J Med*. 1990; 322:1149-51.
- Erian J. Endometrial ablation in the treatment of menorrhagia. *British Journal of Obstetrics & Gynaecology* 1994; 101: 19-22.
- Ewen SP, Sutton CJ. Advantages of laparoscopic supra-cervical hysterectomy. *Baillière's Clin Obstet Gynaecol*. 1995; 9: 707-15.
- Fedele L, Bianchi S, Dorta M, Brioschi D, Dorta M, Brioschi D, Zanotti F, Vercellini P. Transvaginal ultrasonography versus hysteroscopy in the diagnosis of uterine submucous myomas. *Obstet Gynecol* 1991; 77: 745-8.
- Fenech A, Abela GS, Crea F, Smith W, Feldman R, Conti CR. A comparison study of laser beam characteristics in blood and saline media. *Am J Cardiol* 1985; 55: 1389-1392.
- Fehr MK, Wyss P, Tromberg BJ, Krasieva T, DiSaia PJ, Lin F, Tadir Y. Selective photosensitizer localisation in the human endometrium after intrauterine application of 5-aminolevulinic acid. *Am J Obstet Gynecol* 1996; 175: 1253-1259.
- Fehr MK, Madsen SJ, Svaasand LO, Tromberg BJ, Eu-sebio J, Berns MW, Tadir Y. Intrauterine light delivery for photodynamic therapy of the human endometrium. *Hum Reprod* 1995; 10:3067-3072.
- Ferenczy A. Studies on the cytodynamics of human endometrial regeneration. I. Scanning electron microscopy. *Am J Obstet Gynecol* 1976; 124: 64-74.
- Ferenczy A. Studies on the cytodynamics of human endometrial regeneration. II. Transmission electron microscopy and histochemistry. *Am J Obstet Gynecol*. 1976; 124:582-95.
- Ferry J, Farnsworth A, Webster M, Wren B. The efficacy of the pipelle endometrial biopsy in detecting endometrial carcinoma. *Aust N Z J Obstet Gynaecol*. 1993; 33:76-8.
- Finsen, N. (1903). *Phototherapy*: London.
- Fisher, A., Linn Murphree, A. & Gomer, G. Clinical and preclinical photodynamic therapy. *Lasers in Surg Med* 1995; 17: 2-31.
- Fleischer AC, Kalemeris GC, Machin JE, Entman SS, James AE Jr. Sonographic depiction of normal and abnormal endometrium with histopathologic correlation. *Journal of Ultrasound in Medicine* 1986; 5(8): 445-52.
- Fleischer AC, Dudley BS, Entman SS, Baxter JW, Kalemeris GC, James AE Jr. Myometrial invasion by endometrial carcinoma: sonographic assessment. *Radiology*. 1987; 162: 307-10.
-

-
- Fogelman I. The effects of oestrogen deficiency on the skeleton and its prevention. *British Journal of Obstetrics & Gynaecology* 1996; 103: 1-4.
- Foote C. Mechanisms in photooxidation. In *Porphyrin Localisation and Treatment of Tumours*, Doiron, D. & Gomer, C. (eds) 1984; pp. 3-18. Alan R Liss: New York.
- Fraser IS, Pearse C, Shearman RP, Elliott PM, McIlveen J, Markham R. Efficacy of mefenamic acid in patients with a complaint of menorrhagia. *Obstetrics & Gynecology* 1981; 58; 543-51.
- Fraser I, McCarron SG, Markham R. A preliminary study of factors influencing perception of menstrual blood loss volume. *American Journal of Obstetrics & Gynecology* 1984; 149: 788-93.
- Fraser IS. Treatment of dysfunctional uterine bleeding with danazol. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 1985; 25; 224-6.
- Fraser I, McCarron G, Markham R, Resta T. Blood and total fluid content of menstrual discharge. *Obstet Gynecol* 1985; 65: 194-198.
- Fraser IS, McCarron SG, Markham R, Resta T, Watts A., et al. Measured menstrual blood loss in women with menorrhagia associated with pelvic disease or coagulation disorder. *Obstetrics & Gynecology* 1986; 68: 630-3.
- Fraser IS. Menorrhagia due to myometrial hypertrophy: treatment with tamoxifen. *Obstetrics & Gynecology* 1987; 70; 505-6.
- Fraser IS. Treatment of ovulatory and anovulatory dysfunctional uterine bleeding with oral progestogens. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 1990; 30; 353-6.
- Friberg B, Persson BR, Willen R, Ahlgren M. Endometrial destruction by hyperthermia--a possible treatment of menorrhagia. An experimental study. *Acta Obstetrica et Gynecologica Scandinavica* 1996; 75: 330-5.
- Friberg B, Joergensen C, Ahlgren M. Endometrial thermal coagulation--degree of uterine fibrosis predicts treatment outcome. *Gynecol Obstet Invest.* 1998; 45:54-7.
- Gannon MJ, Stringer M, Brown SB. A phase 1 clinical trial of endometrial ablation by ALA-PDT. Conference presentation; 7th Biennial Congress, International Photodynamic Association, 1998, Nantes, France. Abstract no. RC29.
- Gannon MJ. Success of human endometrial PDT. 1998 (Personal communication).
- Gannon MJ, Vernon DI, Holroyd JA, Stringer M, Johnson N, Brown SB. PDT of the endometrium using ALA. *Proceedings of the Society of Photo-optical Instrumentation Engineers* 1997; 2972, 2-13.
- Gannon MJ, Johnson N, Roberts DJ, Holroyd JA, Vernon DI, Brown SB, Lilford RJ. Photosensitization of the endometrium with topical 5-aminolevulinic acid. *Am J Obstet Gynecol* 1995; 173(6):1826-1828.
- Gao J, Ma LY, Zeng S. Menstrual blood loss in healthy Chinese women. *Contraception* 1981; 23: 591-601.
- Gao J. TCM treatment of adolescent functional uterine hemorrhage--a clinical report of 105 cases. *Journal of Traditional Chinese Medicine* 1990; 10; 118-21.
- Garry R, Erian J, Grochmal SA. A multi-centre collaborative study into the treatment of menorrhagia by Nd-YAG laser ablation of the endometrium. *British Journal of Obstetrics & Gynaecology* 1991; 98; 357-62.
- Garry R and Phillips G. How safe is the laparoscopic approach to hysterectomy? *Gynaecological Endoscopy* 1995; 4; 77-79.
- Garry R. Endometrial ablation and resection: validation of a new surgical concept. *British Journal of Obstetrics & Gynaecology* 1997; 104: 1329-31.
-

-
- Garry R. Evidence and techniques in endometrial ablation: consensus. *Gynaecological endoscopy* 2002; 11: 5-17.
- Gath D, Osborn M, Bungay G, Iles S, Day A, Bond A, Passingham C. Psychiatric disorder and gynaecological symptoms in middle aged women: a community survey. *British Medical Journal Clinical Research Ed.* 1987; 294: 213-8.
- Gervaise A, Fernandez H, Capella-Allouc S, Taylor S, Vieille Sla, Hamou J, Gomel V. Thermal balloon ablation versus endometrial resection for the treatment of abnormal uterine bleeding. *Hum Reprod* 1999; 14; 11: 2743-2747.
- Gimpelson RJ, Rappold HO. A comparative study between panoramic hysteroscopy with directed biopsies and dilatation and curettage: a review of 276 cases. *Am J Obstet Gynecol* 1988; 158: 489-92.
- Goh TH, Puvan IS, Wong WP, Sivanesaratnam V, Sinnathuray TA. A study of menstrual patterns following laparoscopic sterilization with silastic rings. *International Journal of Fertility* 1981; 26; 116-9.
- Goldrath MH, Fuller TA, Segal S. Laser photovaporization of endometrium for the treatment of menorrhagia. *American Journal of Obstetrics & Gynecology* 1981; 140: 14-9.
- Goldstein SR, Nachtigall M, Snyder JR, Nachtigall L. Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding. *Am J Obstet Gynecol* 1990; 163: 119-123.
- Gomer C. DNA damage and repair in CHO cells following hematoporphyrin photoradiation. *Cancer Lett.* 1980; 11: 161-7.
- Gomer C & Dougherty T. Determination of [3H] and [14C] hematoporphyrin derivative distribution in malignant and normal tissue. *Cancer Res*, 1979; 39.
- Gomer C, Ferrario A, Hayashi N, Rucker N, Szirth B. & Murphree A. Molecular, cellular, and tissue responses following photodynamic therapy. *Laser Surg Med* 1988; 4: 450-63.
- Granberg S, Wikland M, Karlsson B, Norstrom A, Friberg LG. Endometrial thickness as measured by endovaginal ultrasonography for identifying endometrial abnormality. *Am J Obstet Gynecol* 1991; 164: 47-52.
- Grimes DA. Dilatation and curettage: a reappraisal. *Am J Obstet Gynecol* 1982; 142: 1-6.
- Guillebaud J, Bonnar J, Morehead J, Matthews A. Menstrual blood-loss with intrauterine devices. *Lancet* 1976; 1; 387-90.
- Guillebaud J, Anderson A, Turnbull AC. Reduction by mefenamic acid of increased menstrual blood loss associated with intrauterine contraception. *British Journal of Obstetrics and Gynaecology* 1978; 85: 53-62.
- Guttorm E. Menstrual bleeding with intrauterine contraceptive devices. *Acta Obstetrica et Gynecologica Scandinavica* 1971; 50; 9-16.
- Hahn L, Rybo G. Fibrinogen-fibrin degradation products in menstrual blood from women with normal and excessive menstrual losses. *Acta Obstetrica et Gynecologica Scandinavica* 1975; 54; 119-27.
- Hahn L, Cederblad G, Rybo G, Pehrsson NG, Bengtson KK. Blood coagulation, fibrinolysis and plasma proteins in women with normal and with excessive menstrual blood loss. *British Journal of Obstetrics & Gynaecology* 1976; 83; 974-80.
- Haining RE, Cameron IT, van Papendorp C, Davenport AP, Prentice A, Thomas EJ, Smith SK. Epidermal growth factor in human endometrium: proliferative effects in culture and immunocytochemical localization in normal and endometriotic tissues. *Hum Reprod.* 1991; 6: 1200-5.
-

-
- Hall P, Maclachlan N, Thorn N, Nudd MW, Taylor CG, Garrioch DB. Control of menorrhagia by the cyclo-oxygenase inhibitors naproxen sodium and mefenamic acid. *British Journal of Obstetrics & Gynaecology* 1987; 94: 554-8.
- Hallberg L and Nilsson L. Determination of menstrual blood loss. *Scand J Clin Lab Invest.* 1964; 16: 244-8.
- Hallberg L and Nilsson L. Constancy of individual menstrual blood loss. *Acta Obstet Gynecol Scand.* 1964; 43: 352-9.
- Hallberg L, Hogdahl AM, Nilsson L, Rybo G. Menstrual blood loss - a population study. Variation at different ages and attempts to define normality. *Acta Obstetrica et Gynecologica Scandinavica* 1966; 45: 320-51.
- Harrison RF and Cambell S. A double blind trial of ethamsylate in the treatment of primary and intrauterine-device menorrhagia. *Lancet* 1976 ii:283-285.
- Harrison-Woolrych M, Raine JM. Levonorgestrel intrauterine device can be left in place for five years [letter]. *BMJ* 1998; 317: 149.
- Hart A. Mann-Whitney test is not just a test of medians: differences in spread can be important. *BMJ* 2001; 323: 391-393.
- Haynes PJ, Hodgson H, Anderson AB, Turnbull AC. Measurement of menstrual blood loss in patients complaining of menorrhagia. *British Journal of Obstetrics & Gynaecology* 1977; 84: 763-8.
- Haynes, P. J., A. P. Flint, et al. (1980). Studies in menorrhagia: (a) mefenamic acid, (b) endometrial prostaglandin concentrations. *International Journal of Gynaecology & Obstetrics* 17(6): 567-72.
- Haynes, P. and B. L. Parry (1998). Mood disorders and the reproductive cycle: affective disorders during the menopause and premenstrual dysphoric disorder. *Psychopharmacology Bulletin* 34(3): 313-8.
- Hefnawi, F., A. Saleh, et al. Fibrinolytic activity of menstrual blood in normal and menorrhagic women and in women wearing the Lippes Loop and the Cu-T (200). *International Journal of Gynaecology & Obstetrics* 1979; 16(5): 400-7.
- Henderson, B. W. and D. A. Bellnier (1989). Tissue localization of photosensitizers and the mechanism of photodynamic tissue destruction. *Ciba Foundation Symposium* 146: 112-25; discussion 125-30.
- Henderson, B. W. and V. H. Fingar. Oxygen limitation of direct tumor cell kill during photodynamic treatment of a murine tumor model. *Photochemistry & Photobiology* 1989; 49: 299-304.
- Henderson BW, Dougherty TJ. How does the photodynamic therapy work? *Photochem Photobiol* 1992;55:145-147.
- Hershko C, Link G, Pinson A, Peter HH, Dobbin P, Hider RC (1991). Iron mobilization from myocardial cells by 3-hydroxy-pyridin-4-one chelators: studies in rat heart cells in culture. *Blood* 77: 2049-2053.
- Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. *British Journal of Obstetrics & Gynaecology* 1990; 97: 734-9.
- Higham JM & Shaw RW. The effect of thyroxine replacement on menstrual blood loss in a hypothyroid patient. *British Journal of Obstetrics & Gynaecology* 1992; 99: 695-6.
- Higham JM, Shaw RW. A comparative study of danazol, a regimen of decreasing doses of danazol, and norethindrone in the treatment of objectively proven unexplained menorrhagia. *American Journal of Obstetrics & Gynecology* 1993; 169: 1134-9.
- Hill DJ. Complications of hysterectomy. *Ballière's Clinical Obstetrics and Gynaecology* 1997; 11: 1; 181-197.
-

-
- Hollender MH. A study of patients admitted to a psychiatric hospital after pelvic operations. *Am J Obstet Gynecol.* 1960; 79: 498-503.
- Hollingworth B, Guillebaud J. The levonorgestrel intrauterine device. *The Diplomat* 1994; 1: 247-251.
- Hoyes KP, Porter JB. Subcellular distribution of desferrioxamine and hydroxypyridin-4-one chelators in K562 cells affects chelation of intracellular iron pools. *Br J Haematol* 1993; 85: 393-400.
- Hulka CA, Hall DA, McCarthy K, Simeone JF. Endometrial polyps, hyperplasia, and carcinoma in postmenopausal women: differentiation with endovaginal sonography. *Radiology.* 1994;191:755-8.
- Hulka JF, Peterson HA, Phillips JM, Surrey MW. Operative hysteroscopy: American Association of Gynecologic Laparoscopists' 1993 membership survey. *J Am Assoc Gynecol Laparosc.* 1995; 2: 131-2.
- Hurskainen R, Teperi J, Paavonen J, Cacciatore B. Menorrhagia and uterine artery blood flow. *Human Reproduction* 1999; 14: 186-9.
- Indman PD. Abnormal uterine bleeding: Accuracy of vaginal probe ultrasound in predicting abnormal hysteroscopic findings. *Journal of Reproductive Medicine* 1995; 40: 545-548.
- Irvine GA, Cameron IT. Medical management of dysfunctional uterine bleeding. *Baillieres Best Pract Res Clin Obstet Gynaecol.* 1999; 13: 189-202.
- Irvine GA, Campbell-Brown MB, Lumsden MA, Heikkilä A, Walker JJ, Cameron IT. Randomised comparative trial of the levonorgestrel intrauterine system and norethisterone for the treatment of idiopathic menorrhagia. *Br J Obstet Gynaecol* 1998; 105: 592-598.
- Ishihara S, Taketani Y, Mizuno M. Epidermal growth factor-like immunoreactivity in human endometrium. *Asia Oceania J Obstet Gynaecol.* 1990;16:165-8.
- Jackson P and Lander JL. Female sterilisation: a five-year follow-up in Auckland. *New Zealand Medical Journal* 1980; 91; 140-3.
- Jacobs A, Butler EB. Menstrual blood loss in iron deficiency anaemia. *Lancet* 1965; ii: 407-409.
- Janssen CA, Scholten PC, Heintz AP. A simple visual assessment technique to discriminate between menorrhagia and normal menstrual blood loss. *Obstetrics & Gynecology* 1995; 85; 977-82.
- Jick H, Jick SS, Gurewich V et al. Risk of idiopathic cardiovascular death and non-fatal venous thromboembolism in women using oral contraceptives with different progestagen components. *Lancet* 1995; 346: 1589-1593.
- Johnson LF. Optical maser characteristics of rare earth ions in crystals. *J Appl Phys* 1961; 34: 897-909.
- Jordan PM. Biosynthesis of terapyrroles. In: Neuberger A and van Deenen LLM (Eds). *New Comprehensive Biochemistry.* 1991; Vol 19. pp 1-100. Elsevier Science, New York.
- Judd MD, Bedwell J, MacRobert AJ, Bown SG. Comparison of the distribution of phthalocyanine and ALA-induced porphyrin sensitizers within the rabbit uterus. In: *Photodynamic Therapy and Biomedical Lasers* ed Spinelli P Dal Fante M, Marchesini R, pp 322-326. Elsevier, Amsterdam.
- Kaplan I, Goldman J, Ger R. The treatment of erosions of the uterine cervix by means of the CO₂ Laser. *Obstet Gynecol* 1973; 41: 795-6.
- Karlsson B, Granberg S, Wickland M. et al. Transvaginal ultrasonography of the endometrium in women with post-menopausal bleeding – A Nordic multicenter study. *Am J Obstet Gynecol* 1995; 172: 1488-1494.
-

-
- Kasonde, J. M. and J. Bonnar. Aminocaproic acid and menstrual loss in women using intrauterine devices. *British Medical Journal* 1975; 4: 17-9.
- Kasonde, J. M. and J. Bonnar. Effect of ethamsylate and aminocaproic acid on menstrual blood loss in women using intrauterine devices. *British Medical Journal* 1975; 4: 21-2.
- Kaunitz AM, Masciello A, Ostrowski M, Rovira EZ. Comparison of endometrial biopsy with the endometrial Pipelle and Vabra aspirator. *J Reprod Med.* 1988; 33:427-31.
- Kelly RW, Lumsden MA, Abel MH, Baird D. The relationship between menstrual blood loss and prostaglandin production in the human: evidence for increased availability of arachidonic acid in women suffering from menorrhagia. *Prostaglandins Leukotrienes & Medicine* 1984; 16: 69-78.
- Kelly J. & Snell M. Hematoporphyrin derivative: a possible aid in the diagnosis and therapy of carcinoma of the bladder. *J Urol*, 1976; 115: 150-151.
- Kelly JF, Snell ME, Berenbaum MC. Photodynamic destruction of human bladder carcinoma. *Br J Cancer* 1975; 31: 237-244.
- Kennedy JC, Marcus SL and Pottier RH. Photodynamic therapy and photodiagnosis using endogenous photosensitisation induced by 5-aminolaevulinic acid: mechanisms and clinical results. *J Clin Laser Med Surg* 1996; 14: 289-304.
- Kennedy JC and Pottier RH. Endogenous protoporphyrin IX, a clinical useful photosensitiser for photodynamic therapy. *J Photochem Photobiol B: Biol* 1992; 14: 275-292.
- Kimel S, Tromberg BJ, Roberts WG, Berns MW. Singlet oxygen generation of porphyrins, chlorines and phthalocyanines. *Photochem Photobiol* 1989; 50: 175-183.
- Krampl E, Bourne T, Hurlen-Solbakken H, Istre O. Transvaginal ultrasonography sonohysterography and operative hysteroscopy for the evaluation of abnormal uterine bleeding. *Acta Obstet Gynecol Scand.* 2001 Jul;80(7):616-22.
- Kurjak A, Shalan H, Sosic A, Benic S, Zudenigo D, Kupesic S, Predanic M. Endometrial carcinoma in postmenopausal women: evaluation by transvaginal color Doppler ultrasonography. *Am J Obstet Gynecol.* 1993; 169: 1597-603.
- Kupesic S, Kurjak A. Uterine and ovarian perfusion during the periovulatory period assessed by transvaginal color Doppler. *Fertil Steril.* 1993; 60: 439-43.
- Kupfer MC, Schiller VL, Hansen GC, Tessler FN. Transvaginal sonographic evaluation of endometrial polyps. *J Ultrasound Med.* 1994; 13: 535-9.
- Kyriazis GA, Balin H, Lipson RL. Hematoporphyrin-derivative fluorescent test colposcopy and colpophotography in the diagnosis of atypical metaplasia, dysplasia and carcinoma in situ of the cervix uteri. *Am J Obstet Gynecol* 1973; 117: 375-380.
- Kremer C and Duffy S. Endometrial ablation: the next generation. *British Journal of Obstetrics & Gynaecology* 2000, 107: 1443-1452.
- Kriplani A. Nath J. Takkar D. Maya. Kaul HL. Biochemical hemodynamic and hematological changes during transcervical resection of the endometrium using 1.5% glycine as the irrigating solution. *European Journal of Obstetrics, Gynecology, & Reproductive Biology* 1998; 80: 99-104.
- Lahteenmaki P, Haukkamaa M, Puolakka J et al. Open randomised study of use of levonorgestrel releasing intrauterine system as an alternative to hysterectomy. *BMJ*; 1998; 316: 112-1126.
-

-
- Letchworth AT and Noble AD. Late effects of female sterilisation [letter]. *Lancet* 1977; ii: 768.
- Lewis BV. Hysteroscopy for the investigation of abnormal uterine bleeding. *Br J Obstet Gynaecol.* 1990;97:283-4.
- Lewis BV. Endometrial ablation. *British Journal of Hospital Medicine* 1992; 47: 192-6.
- Lewis BV. Guidelines for endometrial ablation. *British Society of Gynaecological Endoscopy. Br J Obstet Gynaecol.* 1994; 101:470-3.
- Lewis GJ. Do women with menorrhagia need iron? *British Medical Journal Clinical Research Ed.* 1982; 284: 1158.
- Lindoff C, Rybo G, Astedt B. Treatment with tranexamic acid during pregnancy, and the risk of thrombo-embolic complications. *Thrombosis & Haemostasis* 1993; 70; 238-40.
- Lipson, R., Baldes, E. & Olsen, A. (1964a). A further evaluation of the use of hematoporphrin derivative as a new aid for the endoscopic detection of malignant disease. *Dis Chest*, 46, 676.
- Lipson RL, Pratt JH, Baldes EJ, Dockerty MB. Haematoporphyrin derivative for the detection of cervical cancer. *Obstet Gynecol* 1964: 24: 78-84.
- Lipson RL, Gray MJ, Baldes EJ. Haematoporphyrin derivative for the detection and management of cancer. *Proc 9th International Cancer Congress, Tokyo, Japan 1966: 393.* (cited in (Roberts and Cairnduff 1995)
- Lockwood MA, Magos AL, Baumann R, Turnbull AC. Endometrial resection when hysterectomy is undesirable, dangerous or impossible. *British Journal of Obstetrics & Gynaecology* 1990; 97: 656-8.
- Lockwood CJ, Nemerson Y, Krikun G, Hausknecht V, Markiewicz L, Alvarez M, Guller S, Schatz F. Steroid-modulated stromal cell tissue factor expression: a model for the regulation of endometrial hemostasis and menstruation. *J Clin Endocrinol Metab.* 1993;77:1014-9.
- Loft A, Andersen TF, Bronnum-Hansen H, Roepstorff C, Madsen MT. Early postoperative mortality following hysterectomy. A Danish population based study, 1977-1981. *British Journal of Obstetrics & Gynaecology* 1991; 98: 147-54.
- Loffer FD. Three year comparison of thermal balloon and rollerball ablation in treatment of menorrhagia. *J Am Ass Gyn Laparoscopists.* 2001; 8: 48-54.
- Loffer FD. Laser ablation of the endometrium. *Obstet Gynecol Clin North Am* 1988; 15: 77-89.
- Loffer FD. Hysteroscopy with selective endometrial sampling compared with dilatation and curettage for abnormal uterine bleeding the value of a negative hysteroscopic view. *Obstet Gynecol* 1989; 73: 16-20.
- Ludwig H, Frauli M. Immunoprotection of implantation. *Eur J Obstet Gynecol Reprod Biol.* 1990; 36: 249.
- Luukkainen T, Lahteenmaki P, Toivonen J. Levonorgestrel-releasing intrauterine system. *Annals of Medicine* 1990; 22; 85-90.
- Luukkainen T, Toivonen J. Levonorgestrel-releasing IUD as a method of contraception with therapeutic properties. *Contraception* 1995; 52; 269-76.
- MacKenzie I, Bibby J. Critical assessment of dilatation and curettage in 1029 women. *Lancet* 1978; ii: 566-568.
- MacRobert A. Photosensitising compounds. *Ciba foundation symposium*, 1989; 146; 4-16.
- MacRobert AJ. Photodynamic therapy using systemically administered 5-aminolaevulinic acid. *Trends Photochem Photobiol* 1994; 3: 403-412.
-

-
- Magos AL, Baumann R, Turnbull AC. Transcervical resection of endometrium in women with menorrhagia. *BMJ* 1989; 298; 1209.
- Magos AL, Baumann R, Lockwood GM, Turnbull AC. Experience with the first 250 endometrial resections for menorrhagia [published erratum appears in *Lancet* 1991 Jan 1; 337: 1362]. *Lancet* 1991; 337: 1074-8.
- Maiman T. Stimulated optical radiation in ruby. *Nature*, 1960; 187, 493-4.
- Makarainen L, Ylikorkala O. Primary and myoma-associated menorrhagia: role of prostaglandins and effects of ibuprofen. *British Journal of Obstetrics & Gynaecology* 1986; 93; 974-8.
- Makheja AN, Vanderhoek JY, Bailey JM. Inhibition of platelet aggregation and thromboxane synthesis by onion and garlic. *Lancet* 1979; I: 781.
- Markee JE. Menstruation in intraocular endometrial transplants in the rhesus monkey. *Contrib Embryol* 1940; 28: 219-308. (cited).
- Marsh MM, Malakooti N, Taylor NH, Findlay JK, Salamonsen LA. Endothelin and neutral endopeptidase in the endometrium of women with menorrhagia. *Human Reproduction* 1997; 12: 2036-40.
- Martin RL, Roberts WV, Clayton PJ et al. Psychiatric status after hysterectomy. *JAMA* 1980; 244: 350-353.
- Marynissen JP, Jansen H, Star WM. Treatment system for whole bladder wall photodynamic therapy with in vivo monitoring and control of light dose rate and dose. *J Urol*. 1989; 142:1351-5.
- Maas CP, Weijnenborg PT, ter Kuile MM. The effect of hysterectomy on sexual functioning. *Annu Rev Sex Res*. 2003; 14:83-113.
- Maas CP, ter Kuile MM, Laan E, Tuijnman CL, Weijnenborg PT, Tumbos JB, Kenter GG. Objective assessment of sexual arousal in women with a history of hysterectomy. *BJOG* 2004; 111(5):456-62.
- Matthewson K, Coleridge Smith P, O'Sullivan JP, Northfield TC, Bown S. Biological effects of intrahepatic neodymium:yttrium-aluminum-garnet laser photocoagulation in rats. *Gastroenterology* 1987; 93: 550-7.
- McDonagh AF, Lightner DA. Bilirubin, jaundice and phototherapy. *Paediatrics* 1985; 75: 443-455.
- McLennan CE, Rydell AH. Extent of endometrial shedding during normal menstruation. *Obstet Gynecol*. 1965;26:605-21.
- Meikle S, Brody H, Pysh F. An investigation into the psychological effects of hysterectomy. *Journal of Nervous Mental Disorders* 1979; 164: 36-41.
- Meyer WR, Walsh BW, Grainger DA, Peacock LM, Loffer FD, Steege JF. Thermal balloon and rollerball ablation to treat menorrhagia: a multicenter comparison. *Obstetrics & Gynecology* 1998; 92; 98-103.
- Mikuta JJ. Surgical management of dysfunctional uterine bleeding. *Clin Obstet Gynecol*. 1970 Jun;13(2):451-9.
- Milsom I, Andersson K, Andersch B, Rybo G. A comparison of flurbiprofen, tranexamic acid, and a levonorgestrel-releasing intrauterine contraceptive device in the treatment of idiopathic menorrhagia. *American Journal of Obstetrics & Gynecology* 1991; 164: 879-83.
- Moen MD, Webb MJ, Wilson TO, Lee RA. Vaginal hysterectomy in patients with benign uterine enlargement. *Journal of PELVIC Surgery* 1995;1 197-203.
- Mousa HA, Abou El Senoun GM, Mahmood TA. Medium-term clinical outcome of women with menorrhagia treated by rollerball endometrial ablation versus abdominal hysterectomy with conservation of at least one ovary. *Acta Obstetrica et Gynecologica Scandinavica* 2001; 80(5): 442-6.
-

-
- Moyer LD. Endometrial disease in infertility. In: Behrman SJ and Kistner RW (eds) *Progress in infertility*. 1968. Boston, Little, Brown & Co: p132.
- Muldoon MJ. Gynaecological illness after sterilisation. *BMJ* 1972; 1: 84-85.
- NICE (National Institute for Clinical Excellence) Microwave endometrial ablation. August 2003. URL: www.nice.org.uk
- NICE (National Institute for Clinical Excellence) Fluid-filled thermal balloon and microwave endometrial ablation techniques for heavy menstrual bleeding. April 2004. URL: www.nice.org.uk
- Neuwirth RS, Duran AA, Singer A, MacDonald R, Bolduc L. The endometrial ablator: a new instrument. *Obstetrics & Gynecology* 1994; 83: 792-6.
- NHS Centre for Reviews and Disasemination, University of York. The management of menorrhagia. *Effective Health Care* 1: (9).
- Nilsson L, Rybo G. Treatment of menorrhagia. *American Journal of Obstetrics & Gynecology* 1971; 110: 713-20.
- O'Reilly G, Charnock-Jones DS, Davenport AP, Cameron IT, Smith SK. Presence of messenger ribonucleic acid for endothelin-1, endothelin-2, and endothelin-3 in human endometrium and a change in the ratio of ETA and ETB receptor subtype across the menstrual cycle. *J Clin Endocrinol Metab*. 1992; 75: 1545-9.
- Overton C, Hargreaves J, Maresh M. A national survey of the complications of endometrial destruction for menstrual disorders: the MISTLETOE study. Minimally Invasive Surgical Techniques--Laser, EndoThermal or Endorescetion. *British Journal of Obstetrics & Gynaecology*; 1997; 104: 1351-9.
- Parrish JA, Fitzpatrick TB, Tattenbaum L, Pathak PA. Photochemotherapy of psoriasis with oral methoxsalin and long wave ultraviolet light. *N Engl J Med* 1974; 291: 1207-1222.
- Parrish JA, Rosen CF, Gange RW. Therapeutic uses of light. *Ann N Y Acad Sci*. 1985;453:354-64.
- Parys BT, Haylen BT, Hutton JL, Parsons KF. The effects of simple hysterectomy on vesicourethral function. *Br J Urol*. 1989 Dec;64(6):594-9.
- Parys BT. Effect of hysterectomy on bowel function. *BMJ*. 1989; 14:979.
- Patel C. Continuous wave laser action on vibrational-rotational transitions of CO₂. *Phys Rev A*, 1964; 136: 1187-93.
- Pendergrass PB, Scott JN, Ream LJ. A rapid, noninvasive method for evaluation of total menstrual loss. *Gynecol Obstet Invest*. 1984;17(4):174-8.
- Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, Nesland JM. 5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. *Cancer* 1997; 79: 2282- 2308.
- Phipps, J. H., B. V. Lewis, et al. (1990). Experimental and clinical studies with radiofrequency-induced thermal endometrial ablation for functional menorrhagia. *Obstetrics & Gynecology* 76(5 Pt 1): 876-81.
- Phipps, J. H., B. V. Lewis, et al. (1990). Treatment of functional menorrhagia by radiofrequency-induced thermal endometrial ablation. *Lancet* 335(8686): 374-6.
- Pinion SB, Parkin DE, Abramovich DR, Naji A, Alexander DA, Russell IT, Kitchener HC. Randomised trial of hysterectomy, endometrial laser ablation, and transcervical endometrial resection for dysfunctional uterine bleeding. *BMJ* 1994; 309: 979-83.
-

-
- Poad D, Arnold EP. Sexual function after pelvic surgery in women. *Aust N Z J Obstet Gynaecol*. 1994; 34: 471-4.
- Policard, A. Etudes sur les aspects offerts par des tumeurs experimentales examinée a la lumiere de Woods. *C R Soc Biol*, 91, 1423-1428. [Studies of experimental tumours under Wood's light]. (cited in Bonnett 1999).
- Polivy J. Psychological reactions to hysterectomy: a critical review. *Am J Obstet Gynecol*. 1974; 118: 417-26.
- Pokras R, Hufnagel VG. Hysterectomy in the United States 1965–1984. *Am J Public Health* 1988;78:852–853.
- Pope, A. J. and S. G. Bown (1991). The morphological and functional-changes in rat bladder following photodynamic therapy with phthalocyanine photosensitization. *Journal of Urology* 145(5): 1064-1070.
- Pope, A. J. and S. G. Bown (1991). Photodynamic Therapy. *British Journal Of Urology* 68(1): 1-9.
- Pope, A. J., A. J. MacRobert, Bown SG. (1991). The Detection Of Phthalocyanine Fluorescence In Normal Rat Bladder Wall Using Sensitive Digital Imaging Microscopy. *British Journal Of Cancer* 64(5): 875-879.
- Porter JB, Abeysinghe RD, Hoyes KP, Barra C, Huehns ER et al. Contrasting Interspecies Efficacy and Toxicology Of 1,2-Diethyl-3- Hydroxypyridin-4-One, Cp94, Relates to Differing Metabolism Of the Iron-Chelating Site. *British Journal Of Haematology* 1993; 85: 159-168.
- Poulter NR, Chang CL, Farley TMM et al. Venous thromboembolic disease and combined contraceptives: results of international multicentre case-control study. *Lancet* 1995; 346: 1575-1582.
- Prentice A. Health care implications of dysfunctional uterine bleeding. *Baillieres Clinical Obstetrics & Gynaecology* 1999; 13: 181-188.
- Preston JT, Cameron IT, Adams EJ, Smith SK. Comparative study of tranexamic acid and norethisterone in the treatment of ovulatory menorrhagia. *British Journal of Obstetrics & Gynaecology* 1995; 102: 401-6.
- Quick AJ. Menstruation in hereditary bleeding disorders. *Obstet Gynecol* 1966; 28: 37-48.
- Raab O. Ueber die Wirkung fluoreszierenden Stoffe auf Infusorien. *Z Biol* 1900; 39: 524-546. (Cited in Daniell and Hill 1991).
- Rai VS, Gillmer MD, Gray W. Is endometrial pre-treatment of value in improving the outcome of transcervical resection of the endometrium? *Hum Reprod* 2000; 15: 1989-92.
- Razum N, Balchum OJ, Profio AE, Carstens F. Skin photosensitivity: Duration and intensity following intravenous hematoporphyrin derivatives HpD and DHE. *Photochem Photobiol* 1987; 46: 431-438.
- Rees MC, Anderson AB, Demers LM,Turnbull AC. Prostaglandins in menstrual fluid in menorrhagia and dysmenorrhoea. *British Journal of Obstetrics & Gynaecology* 1984; 91; 673-80.
- Rees MC, Cederholm-Williams SA,Turnbull AC. Coagulation factors and fibrinolytic proteins in menstrual fluid collected from normal and menorrhagic women. *British Journal of Obstetrics & Gynaecology* 1985; 92; 1164-8.
- Rees MC, DiMarzo V, Tippins JR, Morris HR,Turnbull AC. Leukotriene release by endometrium and myometrium throughout the menstrual cycle in dysmenorrhoea and menorrhagia. *Journal of Endocrinology* 1987; 113; 291-5.
- Rees MC. Human menstruation and eicosanoids. *Reproduction, Fertility, & Development* 1990; 2; 467-76.
-

-
- Reich H, Ribeiro SC, Vidali A. Hysterectomy as treatment for dysfunctional uterine bleeding. *Baillière's Clinical Obstetrics and Gynaecology* 1999; 13:2; 161-179.
- Reid PC, Coker A, Coltart R. Assessment of menstrual blood loss using a pictorial chart: a validation study. *British Journal of Obstetrics & Gynaecology* 2000; 107: 320-322.
- Ricci JV. Gynaecological surgery and instruments of the nineteenth century prior to the antiseptic age. In: *The development of gynaecologic surgery and instruments*. Philadelphia: Blakiston, 1949: 326-328.
- Rioux JE. Late complications of female sterilization: a review of the literature and a proposal for further research. *J Reprod Med*. 1977; 19(6): 329-40
- Ripley, P. Invited review: The physics of diode lasers. *Lasers Med Sci*. 1996;11, 71-78.
- Roberts, D. J. and F. Cairnduff. Photodynamic therapy of primary skin cancer: a review. *British Journal of Plastic Surgery* 1995; 48: 360-70.
- Roberts RN, Norman BP, Harrison CG, Heaton NR, Law JK, Wadehra V, Younger HM, West CP. A medical audit and patient survey of hysterectomies performed for menstrual disorders. *Scottish Medical Journal* 1996; 41; 44-6.
- Roberts, W. G., K. M. Smith, et al. Skin photosensitivity and photodestruction of several potential photodynamic sensitizers. *Photochemistry & Photobiology* 1989; 49(4): 431-8.
- Rodrigues GC, Yaqub N, King ME. A comparison of the pipelle device and the vabra aspirator as measured by endometrial denudation in hysterectomy specimens: the pipelle device samples significantly less of the endometrial surface than the vabra aspirator. *Am J Obstet Gynecol* 1993; 16: 55-59.
- Rollier A. *Heliotherapy*. Oxford: Oxford University Press, 1927 (cited by Bonnett 1999).
- Romer T. prospective comparison study of levonorgestrel IUD versus Roller-Ball endometrial resection in the management of refractory hypermenorrhea. *Eur J Obstet Gynecol Repro Biol*. 2000; 90(1): 27-9.
- Rosenberg MJ, Foldes R, Mishell DR Jr, Speroff L, Waugh MS, Burkman R. Performance of the TCu380A and Cu-Fix IUDs in an international randomized trial. *Contraception*. 1996; 53:197-203.
- Royal College of Obstetricians and Gynecologists. In-patient treatment - D & C in women age 40 or less. Evidence-based Clinical Guidelines No. 3. Royal College of Obstetricians and Gynecologists, London: 1994
- Royal College of Obstetricians and Gynecologists. The initial management of Menorrhagia. Evidence-based Clinical Guidelines No. 1. The Royal College of Obstetricians and Gynaecologists, London: 1998.
- Royal College of Obstetricians and Gynecologists. The management of Menorrhagia in secondary care. Evidence-based Clinical Guidelines No. 5. The Royal College of Obstetricians and Gynaecologists, London:1999.
- Rubenstein LM, Lebhertz TB, Kleinkopf V. Laparoscopic tubal sterilisation: long-term post operative follow-up. *Contraception* 1976; 13: 631.
- Rybo G, Hallberg L. Influence of hereditary and environment on normal menstrual blood loss. A study of twins. *Acta Obstetrica et Gynecologica Scandinavica* 1966; 45: 389-410.
- Rybo G. Plasminogen activators in the endometrium. II. Clinical aspects. Variation in the concentration of plasminogen activators during the menstrual cycle and its relation to menstrual blood loss. *Acta Obstetrica et Gynecologica Scandinavica* 1966; 45: 429-50.
-

-
- Rybo G. Clinical and experimental studies on menstrual blood loss. *Acta Obstetrica et Gynecologica Scandinavica* 1966; 45(Suppl): 1-23.
- Sahwi S, Toppozada M, Kamel M, Anwar MY, Ismail AA. Changes in menstrual blood loss after four methods of female tubal sterilization. *Contraception* 1989; 40: 387-98.
- Salamonsen LA, Kovacs GT, Findlay JK. Current concepts of the mechanisms of menstruation. *Baillieres Best Pract Res Clin Obstet Gynaecol.* 1999 Jun;13(2):161-79.
- Saxton DW, Farquhar CM, Rae T, Beard RW, Anderson MC, Wadsworth J. Accuracy of ultrasound measurements of female pelvic organs. *Br J Obstet Gynaecol.* 1990; 97: 695-9
- Schenker JG, Polishuk WZ. Regeneration of rabbit endometrium after cryosurgery. *Obstet Gynecol.* 1972;40:638-45.
- Schenker JG, Sacks MI, Polishuk WZ. I. Regeneration of rabbit endometrium following curettage. *Am J Obstet Gynecol.* 1971;111:970-8. N
- Scott JC and Mussey E. Menstrual patterns in myxedema. *Am J Obstet Gynecol* 1979; 301: 460-465.
- Sharp NC, Cronin N, Feldberg I, Evans M, Hodgson D, Ellis S. Microwaves for menorrhagia: a new fast technique for endometrial ablation. *Lancet* 1995; 346; 1003-4.
- Shaw ST (Jnr), Macaulay LK, Sun NC, Tanaka MS (Jnr), Roche PC. Changes of plasminogen activator in human uterine tissue induced by intrauterine contraceptive devices. *Contraception* 1993; 27: 131-40.
- Shearman RP. Secondary amenorrhoea. In: Dewhurst's Textbook of Obstetrics and gynaecology for postgraduates. Whitfield CR (ed.), Fifth edition: 1995; 31-42. Blackwell Science Ltd. Oxford.
- Sheth S, Hamper U, Kurman R. Thickened endometrium in the post-menopausal woman: sonographic-pathologic correlation. *Radiology* 1993; 187: 135-139.
- Short RV. The evolution of human reproduction. *Proc R Soc Lond* 1976; 195: 3-24.
- Sidi E, Bourgeois-Gavardin J. the treatment of vitiligo with *Ammi majus* Linn. *J Invest Dermat* 1952; 18: 391 (cited in Fitzpatrick and Pathak 1958)
- Smith SK, Abel MH, Kelly RW, Baird DT. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. *British Journal of Obstetrics & Gynaecology* 1981; 88: 434-42.
- Smith-Bidman R, Kerlikowske K, Feldstein V et al. Endovaginal ultrasound to exclude endometrial cancer and other endometrial abnormalities. *JAMA* 1998; 280: 1510-1517.
- Smith SK, Abel MH, Kelly RW, Baird DT. A role for prostacyclin (PGI₂) in excessive menstrual bleeding. *Lancet* 1981; i: 522-4.
- Spencer CP, Whitehead MI. Endometrial assessment re-visited. *BJOG* 1999; 106: 623-632.
- Spitzer M and BA Krumholz. Photodynamic therapy in gynecology. *Obstetrics & Gynecology Clinics of North America* 1991; 18: 649-59.
- Steiner RA, Tromberg BJ, Tadir Y, Tromberg BJ, Krasieva T, Ghazains AT, Wyss P, Berns MW. Rat reproductive performance following photodynamic therapy with topically administered Photofrin. *Hum Reprod* 1995; 10: 227-33.
- Steiner RA, Tadir Y, Tromberg BJ, Krasieva T, Ghazains AT, Wyss P, Berns MW. Photosensitization of the rat endometrium following 5-aminolevulinic acid induced photodynamic therapy. *Lasers in Surgery & Medicine* 1996; 18: 301-8.
-

-
- Stock RJ. Evaluation of sequelae of tubal ligation. *Fertility & Sterility* 1978; 29: 169-74.
- Stott PC. The outcome of menorrhagia: a retrospective case control study. *J R Coll Gen Pract* 1983;33:715-720.
- Stovall G, Photopolous G, Poston W, Ling F, Sandles L. Pipelle endometrial sampling in patients with known endometrial carcinoma. *Obstet Gynecol* 1991; 77: 954-956.
- Sturridge F, Guillebaud J. A risk-benefit assessment of the levonorgestrel-releasing intrauterine system. *Drug Safety* 1996; 15: 430-40.
- Stryer L. *Biochemistry*, 3rd edition. WH Freeman: New York. 1998; pp 594-597.
- Svaasand LO, Gomer CJ, Profio AE. Laser induced hyperthermia of ocular tumours. *Appl. Optics* 1989; 28: 2280-2287 (cited in Wyss et al 1995).
- Svaasand LO. Optical dosimetry for direct and interstitial photoradiation therapy of malignant tumors. In: Doiron D, Gomer CJ, editors. *Porphyrin localization and treatment of tumors*. New York: Alan R. Liss, Inc.; 1984. p 91-114.
- Svaasand, L. (1985). Photodynamic and photothermic response of malignant tumours. *Med Phys*, 12, 455-61.
- Syrop C, Sahakian V. Transvaginal sonographic detection of endometrial polyps with fluid contrast augmentation. *Obstet Gynecol* 1992; 79: 1041-1043.
- Tang GWK and Lo SST. Levonorgestrel intrauterine device in the treatment of menorrhagia in Chinese women: efficacy versus acceptability. *Contraception* 1995; 51: 231-235.
- Tanner JM, O'Keeffe B. Age at menarche in Nigerian schoolgirls, with a note on their heights and weights from age 12 to 19. *Hum Biol.* 1962; 34: 187-96.
- Tappeiner V, & Jesionek A. Ueber die Wirkung der photodynamischen (fluorescierenden) Stoffe auf Protozoen und Enzyme. *Arch Klin Med*, 1904; 80: 427-87 (cited in Daniell and Hill 1991).
- Telfer JF, Irvine GA, Kohnen G et al. Expression of endothelial and inducible nitric oxide synthase in non-pregnant and decidualised endometrium. *Molecular Human Reproduction* 1997; 3: 69-75.
- Thacker HL. In postmenopausal women who have had a subtotal (simple) hysterectomy, how much estrogen is enough, and how do you test for deficiency? *Cleve Clin J Med*. 2000 Dec;67(12):877-8.
- Thomas EJ, Okuda KJ, Thomas NM. The combination of a depot gonadotrophin releasing hormone agonist and cyclical hormone replacement therapy for dysfunctional uterine bleeding. *British Journal of Obstetrics & Gynaecology* 1991; 98: 1155-9.
- Towbin NA, Gviazda IM, March CM. Office hysteroscopy versus transvaginal ultrasonography in the evaluation of patients with excessive uterine bleeding. *American Journal of Obstetrics & Gynecology* 1996; 174: 1678-1682.
- Tromberg BJ, Svaasand LO, Fehr MK, Madsen SJ, Wyss P, Sansone B, Tadir Y. A mathematical model for light dosimetry in photodynamic destruction of human endometrium. *Phy Med Biol* 1996; 41: 223-237.
- Treloar SA, Do KA, O'Connor VM, O'Connor DT, Yeo MA, Martin NG. Predictors of hysterectomy: an Australian study. *American Journal of Obstetrics & Gynecology* 1999; 180: 945-54.
- Vancaillie TG. Electrocoagulation of the endometrium with the ball-ended resectoscope. *Obstetrics and Gynaecology* 1989; 74: 425-427.
-

-
- van den Bosch T, Vandendael A, Van Schoubroeck D, Wranz PA, Lombard CJ. Combining vaginal ultrasonography and office endometrial sampling in the diagnosis of endometrial disease in postmenopausal women. *Obstet Gynecol* 1995; 85: 349-52.
- van Eijkeren MA, Scholten PC, Christiaens GC, Alsbach GP, Haspels AA. The alkaline hematin method for measuring menstrual blood loss--a modification and its clinical use in menorrhagia. *European Journal of Obstetrics, Gynecology, & Reproductive Biology* 1986; 22; 345-51.
- van Eijkeren MA, Christiaens GC, Sixma JJ, Haspels AA. Menorrhagia: a review. *Obstetrical & Gynecological Survey* 1989; 44; 421-9.
- van Eijkeren MA, Christiaens GC, Haspels AA, Sixma JJ. Measured menstrual blood loss in women with a bleeding disorder or using oral anticoagulant therapy. *American Journal of Obstetrics & Gynecology* 1990; 162; 1261-3.
- van Geel, I.P., Oppelaar, H., Rijken, P., Bernsen, H., Hagemeier, N., van der Kogel, A. & Stewart, F.A. (1996). Vascular perfusion and hypoxic areas in RIF-1 tumours after photodynamic therapy. *Br J Cancer*, 73, 288-93.
- van Geel, I. P., H. Oppelaar, et al. (1996). Influence of fractionation and fluence rate in photodynamic therapy with Photofrin or mTHPC. *Radiation Research* 145(5): 602-9.
- van Look PF, Lothian H, Hunter WM, Michie EA, Baird DT. Hypothalamic-pituitary-ovarian function in perimenopausal women. *Clin Endocrinol* 1977; 7: 13-31.
- Vasilenko P, Kraicer PF, Kaplan R, deMasi A, Freed N. A new and simple method of measuring menstrual blood loss. *Journal of Reproductive Medicine* 1988; 33; 293-7.
- Vessey MP, Villard-Mackintosh L, McPherson K, Coulter A. The epidemiology of hysterectomy: findings in a large cohort study. *British Journal of Obstetrics & Gynaecology* 1992; 99: 402-7.
- von Tappeiner H, Jesionek A. Therapeutische Versuche mit fluoreszierenden Stoffen. *Muenchener Medizinische Wochenschrift* 1903; 47: 2042-2044. (Cited in Daniell and Hill 1991).
- Wamsteker K, Emanuel MH, de Kruif JH. Transcervical hysteroscopic resection of submucous fibroids for abnormal uterine bleeding results regarding the degree of intramural extension. *Obstet Gynecol* 1993; 82: 736-740.
- Wang L, Jacques S. & Zheng L. Monte Carlo Modelling of light in multilayered tissues. *Comput. Methods. Programs. Biomed* 1995; 47: 131-46.
- Weismann A, Kol S, Peretz B. Gas embolism in obstetrics and gynecology: a review. *J Reprod Med* 1996; 41: 103-111.
- West CP, Lumsden MA, Lawson S, Williamson J, Baird DT. Shrinkage of uterine fibroids during therapy with goserelin (Zoladex): a luteinising hormone-releasing hormone agonist administered as a monthly subcutaneous depot. *Fertil Steril* 1987; 48: 45-51.
- Whitehurst, C., Byrne, K. & Moore, J. (1993). Development of a light source to lasers for photodynamic therapy: 1. *Lasers Med Sci*, 8, 259-67.
- Whitehurst C, Humphries J. & Moore J. Development of a light source to lasers for photodynamic therapy: 2. *Lasers Med Sci*, 1995; 10: 121-6.
- Wilansky DL, Greisman B. Early hypothyroidism in patients with menorrhagia. *American Journal of Obstetrics & Gynecology* 1989; 160; 673-7.
- Wilson, B. & Patterson, M. (1986). Review article: The physics of photodynamic therapy. *Phys Med Biol*, 31, 327-360.
-

-
- Wilson, B., Patterson, M. & Burns, D. (1986). Effect of photosensitizer concentration in tissue on the penetration depth of photoactivating light. *Laser Med Sci*, 1, 235-44.
- Wingo PA, Huelzo CM, Rubin GL, Ory HW, Peterson HB. The mortality risk associated with hysterectomy. *American Journal of Obstetrics & Gynecology* 1985; 152: 803-8
- Witz CA, Silverberg KM, Burns WN, Schenken RS, Olive DL. Complications associated with the absorption of hysteroscopic fluid media. *Fertil Steril*. 1993; 60:745-56.
- Wood C, Hurley VA, Leoni M. The value of vaginal ultrasound in the management of menorrhagia. (1993) *Aust N Z J Obstet Gynaecol* 198-200.
- World Book Multimedia Encyclopedia. Mac OS X Edition (Version 6.0.2). World Book, Inc., 233 N. Michigan Avenue, Suite 2000, Chicago, IL 60601. USA.
- Wyman, D. R., B. C. Wilson, et al. Medical Imaging-Systems For Feedback-Control Of Interstitial Laser Photocoagulation. *Proceedings Of the IEEE* 1992; 80: 890-902.
- Wyss P, Tromberg BJ, Wyss MT, Krasieva T, Schell M, Berns MW, Tadir Y. Photodynamic destruction of endometrial tissue with topical 5-aminolevulinic acid in rats and rabbits. *American Journal of Obstetrics & Gynecology*. 171(5):1176-83, 1994
- Wyss P., Fehr M., Van den Bergh H., Haller U. (1998). Feasibility of photodynamic endometrial ablation without anesthesia. *International Journal of Gynaecology & Obstetrics* 60(3): 287-8.
- Wyss P, Svaasand LO, Tadir Y, Haller U, Berns MW, Wyss MT, Tromberg BJ. Photomedicine of endometrium: experimental concepts. *Hum Reprod* 1995;10:221-226.
- Wyss, P., R. Steiner, et al. Regeneration processes in rabbit endometrium: a photodynamic therapy model. *Human Reproduction* 1996; 11: 1992-7.
- Yang JZ, Van Vugt DA, Kennedy JC, Reid RL. Evidence of lasting functional destruction of the rat endometrium after 5-aminolevulinic acid-induced photodynamic ablation: prevention of implantation. *Am J Obstet Gynecol* 1993;168:995-1001.

APPENDIX 1: CONFERENCE PRESENTATIONS.

International Meetings.

Invited Session Chairman

XVI FIGO World Congress of Gynaecology and Obstetrics, Washington DC. USA.

Conference session chairman

Current thoughts on the management of fibroids. September 2000.

Oral presentations.

XVI FIGO World Congress of Gynaecology and Obstetrics, Washington, DC (USA).
September 2000.

Does photodynamic therapy have a place in the treatment of menorrhagia?

XVI FIGO World Congress of Gynaecology and Obstetrics, Washington, DC (USA).
September 2000.

Successful treatment of uterine fibroids with interstitial laser photocoagulation.

Four Provinces and Juniors in Obstetrics and Gynaecology, Dublin (Éire).
November 1999.

Successful treatment of uterine leiomyomata using interstitial laser photocoagulation.

National Meetings

British Society for Gynaecological Endoscopy, Leeds.
March 2000.

Photodynamic therapy in the rabbit uterus resulting in endometrial destruction using 5-aminolaevulinic acid with the iron chelator CP94.

Conference presentation award.

Royal Society of Medicine, London.
February 2000.

Does photodynamic therapy (PDT) have a place in the treatment of menorrhagia?

British Society for Gynaecological Endoscopy,

Plymouth.

May 1999.

Interstitial laser photocoagulation; a new, safe and effective treatment for small uterine leiomyoma.

British Medical Laser Association,

Liverpool.

May 1999.

A new method of enhancing endometrial destruction in the rabbit uterus by photodynamic therapy (PDT) using ALA combined with the new iron chelator, CP94.

Conference presentation award.

British Medical Laser Association,

Liverpool.

May 1999.

Using interstitial laser photocoagulation (ILP) to treat small uterine fibroids safely and effectively.

Poster presentations.

British Society for Gynaecological Endoscopy, Leeds.

March 2000.

Minimal access ablation of uterine leiomyomata using interstitial laser photocoagulation.

Royal Society of Medicine, London.

February 2000.

Resorption of uterine leiomyomata after interstitial laser photocoagulation.

Blair Bell Research Society, London.

December 1999.

Interstitial laser photocoagulation: a new, safe and effective treatment for uterine leiomyomata.

Blair Bell Research Society, London.

December 1999.

Endometrial ablation in the rabbit uterus by photodynamic therapy (PDT) using 5-aminolaevulinic acid with the iron chelator CP94.

Four Provinces and Juniors in Obstetrics and Gynaecology, Dublin (Éire).

November 1999.

Photodynamic therapy in the rabbit uterus resulting in endometrial destruction using 5-aminolaevulinic acid with the iron chelator CP94.

British Society for Gynaecological Endoscopy, Plymouth.

May 1999.

Enhancing endometrial destruction in the rabbit uterus by ALA-induced photodynamic therapy (PDT) using the new iron chelator, CP94.

British Society for Gynaecological Endoscopy, Plymouth.

May 1999.

A new method for enhancing topical endometrial photosensitisation of the rabbit uterus with ALA - an essential prerequisite for effective photodynamic therapy.

British Medical Laser Association,
Liverpool.
May 1999.

Interstitial laser photocoagulation; a new, safe and effective treatment for small uterine leiomyoma.

British Medical Laser Association,
Liverpool.
May 1999.

Enhanced endometrial ablation in the rabbit uterus by photodynamic therapy (PDT) using ALA combined with the new iron chelator, CP94.

British Medical Laser Association,
Liverpool.
May 1999.

A new method for enhancing topical endometrial photosensitisation of the rabbit uterus with ALA - an essential prerequisite for effective photodynamic therapy.

APPENDIX 2: MANN-WHITNEY U TEST.

An example of the Mann-Whitney U test.

This test is used to analyse results from studies which have compared two different unmatched groups of subjects on a task. It compares results from each group to see if they differ significantly (Hicks 1995:155).

Comparison of knowledge scores (section 2) between 56 qualified midwives (condition 1) and 12 student midwives (condition 2).

Formula:

$$U = n_1 n_2 + \frac{n_x (n_x - 1)}{2} - T_x$$

- U** = Mann-Whitney
 n_1 = number of subjects in condition 1 (56 qualified midwives)
 n_2 = number of subjects in condition 2 (12 student midwives)
 T_x = larger rank total (i.e. 1790 see below)
 n_x = number of subjects in condition in the larger rank total
(condition 1 = 56)
-

- 1 The total score (Σ) and means for each condition must be calculated.
i.e. condition 1 $\Sigma = 1043$ mean score = 18.62
 condition 2 $\Sigma = 267$ mean score = 22.25
 - 2 Taking the whole set of scores together (56 + 12 = 68 scores), they must be ranked giving the rank of 1 to the lowest, 2 to the next lowest etc. Where there are two or more scores the same, use the tied rank
-

procedure. Add up the ranks the scores would have obtained had they been different and divide this number by the number of scores that are the same. Assign this result to all of these scores that are the same.

Example: A score of 11 occurred twice therefore: had they been different they would have been ranked 4 and 5, $(4 + 5)/2 = 4.5$ This is assigned to all the 11 scores see below.

Table A2.1 Frequency distribution of knowledge scores for student and qualified midwives obtained in Section 2.

Scores	Frequency	Ranked scores
3	1	1
4	1	2
8	1	3
11	2	4.5
12	1	6
13	3	8
14	4	11.5
15	1	14
16	1	15
17	2	16.5
18	11	23
19	7	32
20	8	39.5
21	1	44
22	3	46
23	4	49.5
24	6	54.5
25	3	59
26	6	63.5
27	1	67
29	1	68
Total	68	

3 Add the rank totals for each condition separately;

i.e. rank total of condition 1 = 1790

rank total of condition 2 = 545

4 Using the formula above, select the larger rank total i.e 1790

$$U = [(56 \times 12) + 56 (56 + 1)] / 2 - 1790 = 478$$

5 Because there are unequal numbers in each condition it is necessary to repeat the calculations for the smaller rank total.

i.e.

$$U = [(56 \times 12) + 12 (12 + 1)] / 2 - 545 = 205$$

6 The smaller U value from the two calculations is used to assess the level of significance, by referring to the relevant probability tables.

i.e.

$$U = 205 \quad p = 0.03$$

(a significant result since the **p** value is less than 5%).